TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35.U.S.C. 371

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U.S. APPLICATION NO. 60/073,651, 60/073,652, 60/073,653

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/02493	February 4, 1999	February 4, 1998

TITLE OF INVENTION
CHEMICAL SENSORS HAVING MICROFLOW SYSTEMS AND SENSING SYSTEMS THEREOF HAVING INCREASED STABILITY AND USEFUL LIFE

APPLICANT(S) FOR DO/EO/US
Bruce Towe

Applicant herewith submits to the United States Designated /Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [x] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [x] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [x] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [x] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [x] have been transmitted by the International Bureau
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [x] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [x] Other items or information: Verified Statement Claiming Small Entity Status

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/02493	February 4, 1999	

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17. [ ] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
Basic National Fee (37 CFR 1.492(a)(1)-(5):		
Search Report has been prepared by the EPO or JPO $910.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $700.00		
No international preliminary examination fee paid to USTPO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $770.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... $ 96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$96.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] **20** [ ] **30** months from the earliest claimed priority date (37 C.F.R. 1.492(e)).	$	

Claims	**Number Filed**	**Number Extra**	**Rate**	$	
Total Claims	20 -20=		X $ 22.00	$0	
Independent Claims	1 -3=		X $ 80.00	$0	
Multiple dependent claim(s) (if applicable)			+ $260.00	$0	
TOTAL OF ABOVE CALCULATIONS =				$96.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$48.00	
SUBTOTAL =				$48.00	
Processing fee of $130.00 for furnishing the English translation later than [ ] **20** [ ] **30** months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$48.00	
				Amt.refunded	$
				charged	$

a. [x] A check in the amount of $ 48.00 to cover the above fees is enclosed.

b. [ ] Please charge our Deposit Account No. 02-4377 in amount of $___ to cover the above fees. A copy of this sheet is enclosed.

c. [x] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4377. A copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Signature

July 25, 2000
Date

32,689
Registration No.

Attorney's Docket No. A31178 PCT USA-A 072448.0285
Baker Botts L.L.P.

Applicant or Patentee: Bruce Towe
Serial or Patent No.: TBA Filed or Issued: Herewith
For: CHEMICAL SENSORS HAVING MICROFLOW SYSTEMS AND SENSING SYSTEMS THEREOF HAVING INCREASED STABILITY AND USEFUL LIFE

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION Arizona State University

ADDRESS OF ORGANIZATION Tempe, Arizona 85287-6106

TYPE OF ORGANIZATION University

[X] UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
[ ] TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE]26 USC 501(a) and 501(c)(3))
[ ] NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE __)
(CITATION OF STATUTE __)
[ ] WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
[ ] WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE __)
(CITATION OF STATUTE __)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled CHEMICAL SENSORS HAVING MICROFLOW SYSTEMS AND SENSING SYSTEMS THEREOF HAVING INCREASED STABILITY AND USEFUL LIFE by inventor(s) Bruce Towe described in

[X] the specification filed herewith
[ ] Application Serial No. __, filed __.
[ ] Patent No. __, issued __.

I hereby declare that the rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who

could not qualify as a small business concern under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *Note: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Alan Poskanzer

TITLE IN ORGANIZATION Technology Collaborations & Licensing Officer

ADDRESS OF PERSON SIGNING Arizona State University, P.O. Box 87351, Tempe, AZ 87287-3511

SIGNATURE _____ DATE 7/24/00

CHEMICAL SENSORS HAVING MICROFLOW SYSTEMS AND SENSING SYSTEMS THEREOF HAVING INCREASE STABILITY AND USEFUL LIFE

SPECIFICATION

CROSS-REFERENCE TO RELATED APPLICATION

This nonprovisional application claims priority to United States Provisional Patent Application No. 60/073,651 filed February 4, 1998, United States Provisional Patent Application No. 60/073,652 filed February 4, 1998, and United States Provisional Patent Application No. 60/073,653 filed February 4, 1998, which are all incorporated by reference herein.

TECHNICAL FIELD

The present invention relates generally to chemical sensors used to measure the concentration of a specific chemical or gas dissolved within a fluid, and more particularly, to biochemical sensors and continuous monitoring systems including these sensors which have increased stability and longer useful life.

BACKGROUND OF THE INVENTION

There has been a well recognized need in the scientific and medical arts to accurately and continuously measure the concentration of a chemical dissolved within a fluid. Such continuous monitoring techniques are of great interest in medical applications to monitor the concentration of a chemical in biological environments, e.g., monitoring glucose levels in the blood to treat diabetes. It is also of particular interest in biotechnology applications to maintain and control specific concentration levels of nutrients, such as glucose, in cell culture reactors. In addition, such sensing technologies must have long-term stability in order to

provide feedback information needed to control computerized delivery systems so that a particular chemical can be maintained within preset limits.

The concentration of a specific chemical, such as glucose, in a solution can be measured in a variety of ways. For example, benchtop instruments and clinical automated analyzers often use dye-based color spectrophotometric tests for glucose and other biochemicals. A commonly used color test for glucose is the Trinder test, which is commercially available from the at the Sigma Chemical Co., located in St. Louis, Missouri. This test relies on a reaction between the enzyme glucose oxidase, ambient oxygen, and glucose in a sample to provide a hydrogen peroxide intermediate which initiates a color change in an associated dye system. A sample of blood plasma is introduced into a cuvette containing the reagent, and the glucose concentration is measured by correlating the resulting change in spectrophotometer absorbance at 500 nm to absorbance readings of known glucose concentration standards.

Unfortunately, most of the present benchtop or autoanalyzer methods for measuring the concentration of chemicals, such as glucose, are not suited to continuous monitoring in protein-bearing environments. Commercially available benchtop instruments can typically only measure a single processed sample at a time. Furthermore, these devices either (i) require the user to calibrate the instrument before use, which is labor and time intensive, or (ii) include automatic self-calibration mechanisms, typically before each measurement, which introduces added complexity to the instrument. Thus, there is a need for low maintenance sensors and sensing systems which are simple and can accurately and continuously measure many different chemicals in cell culture reactors and medical applications.

There are also a variety of disposable tests based on colorimetry and amperometry that can be utilized when measuring single desired chemical concentrations in samples by using enzymes. These monitoring methods, however, are not suitable for continuous monitoring since monitoring must be performed manually on a periodic basis. Indeed, there exist relatively few electrode probes

which can continuously monitor bioreactor concentrations of chemicals (e.g., glucose, glutamine, glutamate, urea, and ammonia) for time intervals as long as a week without frequent recalibration or maintenance.

Several attempts have been made to develop electrode probes. These monitoring technologies include enzyme-based methods, which are perhaps the most commonly used approaches to biochemical sensing because they have a high degree of specificity to a given target-chemical (e.g., glucose) and are not subject to inaccurate readings caused by the presence of other chemicals in the solution environment (e.g., blood, culture media, or other test environments). These include an enzyme probe, as described in Guilbault, "Future of Biomembrane Probes," Theory, Design and Biomedical Applications of Solid State chemical Sensors, pp. 193-204, CRC Press 1978; a polarographic electrode with the enzyme glucose oxidase immobilized thereon, as described in Thevenot, "Problems in Adapting a Glucose-Oxidase Electrochemical Sensor into an Implantable Glucose-Sensing Device, Diabetes Care, Vol. 5, No. 3, May-June 1982, pp. 184-189; a biochemical temperature sensing analyzer, as described in U.S. Patent No. 3,878,049, issued to Tannenbaum et al.; a flow-through type thermal detector including a pair of parallel fluid flow paths wherein one of these paths includes a reactor column having an enzyme immobilized on the surface of small glass beads packed in the column as described in U.S. Patent No. 3,972,681, issued to Clack et al.; a heat sensor disposed within a flow path wherein an enzyme is immobilized within a packed column of glass, as described in U.S. Patent No. 4,021,307, issued to Mosbach, all of the forgoing references being incorporated herein. However, all of these sensors are unstable, impractical for continuous monitoring, or employ thermistors which are undesirable due to self-heating and the formation of electric fields. Some of these problems with thermisters have been discussed in Fulton,et al., "Thermal Enzyme Probe with Differential Temperature Measurements in a Laminar Flow-Through Cell," Analytical Chemistry, vol. 52, No. 3, March 1980, pp. 505-508.

Yet another problem with thermal enzyme monitoring technologies employing enzyme coated and non-enzyme coated probes is that, even in a well stirred solution, thermal eddies exist which can give rise to an apparent temperature differential between the enzyme-coated and non-enzyme coated probes irrespective of the concentration of the chemical under test. Such thermal eddies may give rise to false indications of concentrations of the chemical under test, or alternatively, may tend to offset temperature differentials which would otherwise be present due to the chemical reaction of the chemical under test.

Perhaps the most stringent requirements placed on sensors are those intended for biomedical implantable applications. Attempts at implantable sensors for glucose have been under development for nearly thirty years, stemming from the work of Leland Clark in the 1960's. It is recognized that a successful implantable sensor would relieve the diabetic from the constant chore of pricking himself to obtain blood for external testing. However the difficulties and constraints on the performance of implantable sensors are particularly severe. They must (i) be compact enough for implantation into the body; (ii) be stable for a long time period (e.g., about a year); and (iii) operate successfully in the hostile environment in the body. Long-term sensor stability is particularly needed for the ultimate planned use of computer feedback control of insulin delivery systems to maintain the diabetic's blood glucose level within preset limits.

There have been a variety of glucose sensor approaches for potential implantation, but as of this date most have not been implemented since they lack the required stability and longevity needed for implantation. Many of these sensors generally rely on well known polarographic principles that are adapted in various ways for implantation. To be successful, a totally implantable sensor should be operable for at least a year and desirably longer.

One approach, which addresses most of the problems discussed hereinbefore, is the thermopile based calorimetric biochemical sensor described in U.S. Patent No. 4,935,534, issued to Guilbeau et al., which is incorporated herein

by reference. These sensors employ an enzyme as a method of catalyzing a reaction producing a local exothermic heat of reaction which is indicative of the concentration of the substrate-chemical. However, enzyme degradation can reach a threshold where the catalyzed chemical reactions fail to produce the nominal heat of the reaction. As a result, the sensor loses its calibration and its sensitivity to the target-chemical with continued enzyme degradation. High loadings of the enzyme and selective membranes placed over the enzyme can slow the loss of sensor sensitivity. However, the sensor will ultimately become inoperable.

Accordingly, decay of the sensing substance, e.g., enzyme, is a major lifetime limiting factor for implantable sensors. For example, enzyme decay generally limits the lifetime of any implantable enzyme-based sensor system to a duration of typically less than about a month. Such a short sensor life makes long-term implantation impractical, since the patient would have to undergo unnecessary surgery to frequently remove or replace the sensor.

In an effort to avoid the problems of total implantation, researchers have proposed transcutaneous sensors employing microdialysis principles for sampling tissue glucose, as described in Keck et al., "Combination of Microdialysis and Glucosensor Permits Continuous (on line) s.c. Glucose Monitoring in a Patient Operated Device: I In Vitro Evaluation," Horm. Metab. Res., 23:617-18, 1991, Keck et al.,"Combination of Microdialysis and Glucosensor Permits Continuous (on line) SC Glucose Monitoring in a Patient Operated Device: II Evaluation in Animals," Horm. Metab. Res., 24:492-93, 1992, Mascini, "Biosensors for Medical Applications," Sensors and Actuators, 6:79-82, 1992, Mascini et al., "In Vivo Continuous Monitoring of Glucose by Microdialysis Technique in the Monitoring of Subcutaneous Tissue Glucose Concentration," Int. J. Art. Org., 16(5):268-75, 1993, which are all incorporated herein by reference. Microdialysis is a method of sampling the biochemical environment of complex fluids, such as in tissue and blood has been known since the 1980's. It is a process where a sweep fluid is passed through a thin membrane hollow fiber (also referred to as "microdialysis

fiber") that is in contact with the solution being tested. These fibers are well characterized in terms of biocompatibility as a result of their use in kidney dialysis. They are typically made of biocompatible materials, such as cellulose acetate, polysulfone, and polyacrylonitrile, usually in the form of hollow tubes on the order of 200 microns in diameter.

The membrane qualities of the hollow fiber allow the transport of glucose and other low molecular weight materials (typically below about 10,000 wt.) across its walls and so, given sufficient time, a sweep fluid passing through the fiber will achieve diffusive equilibrium with the environment. Proteins, red hemoglobin, and blood formed elements in which the fiber is immersed are excluded from the sweep fluid. The emerging sweep fluid from the fiber is actually a dialysate having a clear color. The dialysate has the same concentration of glucose as the environment as long as the flow velocity of the sweep fluid within the fiber is low enough to permit time for equilibrium.

Transcutaneous approaches to blood glucose measurement by microdialysis have typically interfaced the microdialysis fiber with variations of the enzyme-amperometric glucose sensor. However, this approach also requires the periodic replacement of the sensor as the enzyme decays. Furthermore, these kinds of sensor are notorious for their long-term instability.

Similar to the measurement of a chemical in a fluid, the intravascular measurement of the concentration of gases, such as oxygen and carbon dioxide, is of major interest in clinical diagnostics. Such measurements are particularly valuable in intensive care settings in the hospital and during surgery. Although many techniques of measuring these gases are currently available, there is a particular need for long-term stable and compact sensors. Long-term sensor stability is particularly needed in cell culture bioreactors that employ computer feedback control systems to maintain oxygen tension in cultures within preset limits.

Commercial oxygen sensors for continuously measuring oxygen tension typically use polarographic oxygen electrodes of the Clark electrode type. This type of sensor has poor long-term stability. As a result, these sensors require frequent recalibration when exposed to protein-bearing solutions, such as the blood or bioreactor media.

Commercial carbon dioxide sensors widely use the Severinghaus principle whereby a change in the pH of a bicarbonate buffer solution entrapped behind a gas-permeable membrane is measured and then correlated to gas concentration. These sensors typically suffer from long-term drift and slow response time when exposed to blood or cell culture media. They are generally used in automated instruments of self-cleaning and self-calibrating before each measurement.

Fiber optic catheter-type gas sensors for oxygen and carbon dioxide have been receiving increased attention due to their potentially small sensing tip and rugged construction, as described in Wolfbeis, et al., "Fiber Optic Fluorosensor for Oxygen and Carbon Dioxide," Anal. Chem., 60:2018 (1988); Wolfbeis et al., "Recent Progress in Optical Sensor Design," Proc. SPIE, 906:42 (1988); Wolfbeis edition, Fiber Optic Chemical Sensors and Biosensors, Vol. 2, CRC Press, Boston (1991); Trettnak, "Fiber optic Glucose Sensors with an Oxygen Optrode as a Transducer," Analyst, 113:1519 (1988); Gehrich et al., "Optical Fluorescence and Its Application to an Intravascular Blood Gas Monitoring System," IEEE Trans. Biomed. Engr., Vol. BME-33, No. 2, pp. 117-132, (1986); and Peterson et al., "Fiber Optic Probe for In-Vitro Measurement of Oxygen Partial Pressure," Anal. Chem., 56:62-67 (1984); which are all incorporated herein by reference. Fiber optic type sensors, however, have been reported to exhibit problems when used in blood or complex protein bearing solutions, thereby requiring frequent recalibration.

Fiber optic gas sensors typically use silicone or TEFLON membranes to entrap a small, fixed quantity of a gas sensitive reagent at the tip of the fiber. Due to the limited amount of reagent that can be practically immobilized at the end of

the fiber, these gas sensors are susceptible to problems of leaching, dilution, and gradual loss of activity of the reagent over a short period time, typically just a few days. Other problems are caused by photobleaching of the fixed amount of reagent which is immobilized on the fiber tip. Photobleaching is caused by repeated exposure to intense ultraviolet color excitation from quartz or mercury arc lamps, which are typically used in photodetection systems. Yet another problem is the lack of a sufficient amount of light ultimately entering the photodetector at the remote end of the fiber. This inadequate amount of transmittance can contribute to sensor signal-to-noise ratio problems.

SUMMARY OF THE INVENTION

It is, therefore, an object of the present invention to provide a long-term stable, continuously monitoring, sensing system for measuring the concentration of chemical species in a fluid.

It is a further object of the present invention to provide a long-term stable, continuously monitoring, sensing system for measuring the concentration of gas species in a fluid.

These and other objects of the invention are achieved by providing a sensing system for detecting the presence of a target chemical in fluid which is implantable and exhibits long term stability. The system includes a micro-flow reservoir system having at least one micro-flow reservoir including a reagent fluid which reacts with the target chemical and a sensor for detecting the occurrence of the reaction connected to the micro-reservoir system by a conduit which conveys fluid from the micro-flow reservoir system to the sensor. The sensing system can be implanted in a sample host.

The sensor includes a thermopile which measures heat resulting from the reaction of the reagent and target chemical. In an alternate embodiment the sensor includes an optical cell which measures a change in the optical properties of the reagent when contacted with the target chemical.

This arrangement has been found to be useful as a biochemical sensor for use in continuous monitoring systems requiring sensing systems which are stable over long time periods and capable of measuring target chemicals including gases in a fluid.

BRIEF DESCRIPTION OF THE DRAWINGS

Further objects and advantages of the present invention will be more fully appreciated from a reading of the detailed description when considered with the accompanying drawings wherein:

Figure 1 is a schematic of a sensing system including a thermopile in accordance with the invention;

Figure 2A is an illustration of a top view of a containment system in a micro-flow reservoir of the sensing system in accordance with the invention;

Figure 2B is an illustration of a side view of a micro-flow reservoir including multiple collapsible bags in accordance with the invention;

Figure 3 is a schematic of a thermopile used in a sensing system in accordance with the invention;

Figure 4 is an illustration of an implantable sensing system in accordance with the invention wherein the reservoir is refillable through a septum and remote from the sensor;

Figure 5 is a schematic of a sensing system including an optical cell in accordance with the invention;

Figure 6 is a schematic of a sensing system for detecting the presence of a gas in accordance with the invention;

Figure 7 is an illustration of an optical cell for use in a sensing system in accordance with the present invention;

Figure 8 is a schematic of an implantable microfluidic glucose sensor in accordance with the invention;

Figure 9 is a schematic of a sensing arrangement including a vascular graft in accordance with the invention;

Figure 10A and 10B are illustrations of vascular graft arrangements for use in the invention;

Figure 11 is a graph illustrating the correspondence of measured glucose concentration versus true blood glucose concentration for a sensing system having a vascular graft in accordance with the invention;

Figure 12 is a graph of extraction fraction of glucose versus residence time for a sensing arrangement including a vascular graft in accordance with the invention;

Figure 13 is a graph illustrating absorbance versus different mixing ratios of Trinder reagent and glucose at different concentrations;

Figure 14 is a graph of fraction of glucose in a dialysate versus test media as a function of perfusate residence time;

Figure 15 is a graph of output voltage from a photodetector versus CO_2 concentration; and

Figure 16 is a graph of output voltage from a photodetector versus glucose concentration.

DETAILED DESCRIPTION OF THE INVENTION

The sensing systems of the present invention combine various sensor technologies with a means for renewing or replacing the sensing-substance which eventually decays within a short period of time, typically within a week or two months. Sensing-substance, as used herein, means any substance which is needed to facilitate a measurable reaction with the target chemical. Target chemical, as used herein, means the particular chemical, biochemical, or gas for which the concentration measurement is being made. The sensing-substance can be an

enzyme, a dye or any other substance known in the art to facilitate a measurable reaction with the target chemical.

Because the sensing-substance is renewed or replaced, the sensing systems of the present invention exhibit long-term stability. Long-term stability, as used herein, means (i) continuous operation for greater than about 5 months, preferably greater than about 6, more preferably greater than about 8 months, and most preferably greater than about 12 months, without replacing the sensor or replenishing the sensing-substance, and (ii) minimized long-term drift in measurement readings, preferably less than 30%, more preferably less than 25%, most preferably less than 10%.

The long-term stable, chemical and gas sensing systems of the present invention include (i) a micro-flow reagent reservoir, (ii) a sensor fluidly connected to the micro-flow reagent reservoir, and optionally (iii) a waste reservoir fluidly connected to the sensor.

A sensing system in accordance with the invention is shown in Figure 1. In this embodiment the sensing system includes a micro-flow reservoir system **10**, a sensor system **80A** fluidly connected to the micro-flow reservoir system **10** and a waste reservoir **140** fluidly connected to the sensor system **80A**. The sensing system is immersed or implanted in a sample host, for example glucose containing blood. The micro-flow reservoir system includes a reservoir **10a** which contains fresh reagents, and a reservoir **10b** including calibration solutions needed for calibrating the sensor system **80A**. Each micro-flow reservoir may also contain a sweep solution. Each micro-flow reservoir is capable of providing extremely low but continuous flow rates, preferably from about 1 nanoliter/min. to about 1 microliter/min, more preferable from about 10 nanoliter/min. to about 100 nanoliter/min. These micro-flow rates allow the sensing arrangement **80A** to be operational for a long period of time, e.g., greater than 5 months. The flow rate for a specific reagent, calibration solution, and/or sweep solution will depend on the

specific requirements of a particular chemical or gas sensor, as described hereinafter.

Although any micro-flow system well known to one skilled in the art may be used so long as it is capable of providing extremely low but continuous flow rates (e.g., on the order of nanoliters/min.), several nonlimiting embodiments are provided hereinafter.

An exploded view of micro-flow reagent reservoir **10a** is shown in Fig. 2B. As can be seen from Fig. 2B the micro-flow reagent reservoir **10a** includes housing or containment **10c** and a collapsible bag **20** for holding the reagent solution. A tubing arrangement has an open ended tubing portion **30** arranged in a curled position inside the bag **20** and tubing portion **50** located outside the bag **20** which connects the reservoir **10a** to the sensor system **80A**. In an alternate arrangement the tubing portion **30** may be wrapped as a coil around a collapsible bag and housed within the containment.

Returning to Fig. 1 the reservoir 10b includes an additional reagent bag **21** that contains a calibration solution **70** which is insensitive to the concentration of the target-chemical, thereby allowing calibration of the sensor. For example, the reagent bag may contain a dilute solution of hydrogen peroxide that, when perfused through the membrane fiber **90**, will locally diffuse out of it and come into contact with the catalase activity in blood thereby producing a heat of known value. This heat effect will allow calibration of the thermopile **80** and verification of its operation.

The reservoirs **10a** and **10b** can be pressurized with an inert gas, such as nitrogen or a fluorocarbon propellant, such as those used in commercial aerosol dispensers for paint. The collapsible bags can be made of any inert polymeric material known to one skilled in the art, e.g., MYLAR or TEFLON. The collapsible bags **20**, **21** are fluidly connected at the open end to resistance tubing **30** which is typically made of an inert and impermeable material, e.g., silica based capillary microbore tubing, which is commercially available from Polymicro

Technologies, located in Phoenix, Arizona. Microbore tubing is understood by those skilled in the art to be tubing having an inner diameter of less than about 100 μm. Typically, the tubing arrangement has an inner diameter of about 25 microns or less and a length on the order of about one meter. By controlling the charging pressures of reservoirs **10a** and **10b** and the length of the curled portion of the resistance tubing **30** within the collapsible bags **20** and **21**, continuous micro-flow rates on the order of nanoliters/min can be achieved.

An enzyme dissolved in a preservative solution or preserved using any of the methods well known to the state of the art, such as loading the enzyme into microspheres, which is commercially available from Polysciences, Inc., located in Warrington, Pennsylvania, or Sigma Chemical Co., located in St. Louis, Missouri, may be used to provide the reagent solution **60** for reservoir **10a** . This chemical system **60** is then loaded into the collapsible bag **20**, hereinbefore described. This bag communicates with resistance tubing **30**, typically having an inner diameter of 25 microns or less and a length on the order of one meter. With these dimensions for the resistance tubing **30** and a charging pressure of 10 psi for the self-enclosed container **10a**, flow rates on the order of 10 nl per minute can be achieved at the resistance tubing **30** outlet. The outlet of the resistance tubing **30** is in fluid communication, via impermeable microbore tubing **50**, with sensing arrangement **80A**.

The sensor system **80A** includes a hollow membrane fiber **90**, typically having an inner diameter of about 200 microns in diameter, bonded, typically with silicone adhesive or other bonding material well known in the art, onto the surface of a thermopile sensor **80** in place of an enzyme gel or other process which normally entraps a fixed amount of enzyme. These membrane fibers are well known in the art as microdialysis tubing, which is commercially available from Enka, Inc., Scientific Systems, located in Lexington, Massachusetts. Being semipermeable, these hollow membrane fibers have a specific molecular weight cutoff (typically below about 10,000 wt. by weight average) which prevents the

enzyme solution from diffusing out while at the same time allowing glucose and other small molecules to diffuse into the membrane fiber from the test sample, for example blood plasma can diffuse through the membrane fibers. Depending on the thermopile size, two or more loops of the hollow fiber may be needed to completely cover the thermopile sensing junctions, i.e., **100**, **102**, **104**, **106**, **108**, **112**, and **114** as shown in Fig. 3. The membrane hollow fibers used are preferably among those well characterized in terms of their blood biocompatibility and typically made of materials such as cellulose acetate, polysulfone, and polyacrylonitrile, usually in the form of hollow tubes having an inner diameter of about of 200 microns.

Upon contact of the target sample with the reagent solution a chemical reaction ensues within the lumen of the membrane fiber **90**, and the thermopile **80** senses the reaction heat. The thermopile sensor **80** of the type described herein may use a reagent fluid including the enzyme glucose oxidase to catalyze the chemical reaction of glucose and may further include the enzyme catalase for causing the further chemical decomposition of hydrogen peroxide into water and oxygen gas, resulting in the liberation of additional amounts of heat. Such a sensor may also utilize a reagent fluid including enzymes and any associated co-factors, as listed below, in order to detect and measure concentrations of the corresponding chemicals:

1. the enzyme hexokinase (and co-factor ATP) for detecting glucose concentrations;

2. the enzyme glucose dehydrogenase (and co-factor NADP) for detecting glucose concentrations;

3. the enzyme cholesterol oxidase for measuring concentrations of cholesterol;

4. the enzyme lactase for measuring concentrations of lactose;

5. the enzyme urate oxidase for measuring concentrations of uric acid;

6. the enzyme trypsin for measuring concentrations of benzoyl-l-arginine ethyl ester;

7. the enzyme apyrase for measuring enzymatic hydrolysis of ATP into AMP; and

8. the enzyme penicillinase for measuring concentrations of penicillin.

Suitable thermopiles for use in the invention include, but are not limited to, the thermopiles described in U.S. Patent No. 4,935,345 issued to Guilbeau et al., which is incorporated by reference herein. An exploded view of the thermopile **80** is shown in Fig. 3. As illustrated in Fig. 3, this thin film thermopile **80** functions by producing a differential voltage induced by a temperature differential between the sensing junctions (e.g., **100**, **102**, **104**, **106**, **108**, **112**, and **114**) and the reference junctions (e.g., **101**, **103**, **105**, **107**, **109**, and **113**). Thus, the heat of reaction, or the heat of metabolism, resulting from the reaction of the sensing-substance and the target-chemical initiated in the vicinity of the sensing junctions creates a temperature differential between the sensing junctions and the reference junctions which induces a differential voltage thereacross. Appropriate lead wires are connected to the output terminals of the thermopile **110** and **111** for providing the voltage difference signal to amplifiers **120** and **121** and then to the microcontroller **130** which associates the magnitude of the differential voltage to a concentration of the target chemical. The advantage of this approach is that the membrane fibers can be made part of a system which introduces fresh enzyme so that high enzyme activity is maintained.

This system replenishes the enzyme to the reaction zone by providing new enzyme solution into membrane fiber at extremely low but uninterrupted flow rates. The flow is chosen to be the minimum which is just sufficient to offset the decay rate of the enzyme. This varies according to a particular enzyme, but for glucose oxidase can be in the range of about 10 nanoliters per minute, depending on the method of preserving the enzyme activity. This flow rate allows a 5 ml reservoir of enzyme to supply the sensor for nearly a year of operation. The exit of

the membrane fiber can be returned by microbore tubing to recover the decayed chemical system for storage within a separate compartment having a lower pressure than the supply reservoir, or alternatively because of its extremely low flow rate, released into a waste reservoir **140**.

In an alternate embodiment, more than one pressurized reservoir **10a** and **10b** may be employed. Each reservoir may then be charged with a different gas pressure, thereby allowing an alternative method to control the micro-flow rates.

In an additional alternate embodiment shown in Fig. 2b, the micro-flow reagent reservoir may contain multiple bags **20** of different kinds of chemical systems which allow the sensor to measure other target-chemicals by flowing the reactive-substance, e.g., an enzyme or reactive dye, through the microdialysis tubing attached to the sensor. Alternatively, the additional reagent bags can be placed in a separate self-enclosed pressurized reservoir.

In the above embodiments which have multiple reagent bags, microvalves **40**, which are commercially available from TiNi Alloy Co., located in San Leandro, California, are employed to switch between the various reagent bags so as to supply the sensor with a different chemical system. These microvalves are in electrical communication with the computer micro-controller.

The micro-flow reagent reservoir may be remote from the sensor and connected to it by a catheter containing microbore tubing. This system may desirably take the form of a small storage reservoir which contains a means for refilling it in the reservoir. A typical system is illustrated in Figure 4. The reservoir containing the enzyme or chemical system is remote in location from the sensor **220** and connected to it by a catheter **210** of suitable length containing the microbore tubing and electrical lead wires. This system may desirably take the form of a small storage reservoir **200**, perhaps of the size and shape of a pacemaker, which contains a means for refilling by way of a syringe needle through a septa **230** in the containment. This system may be refilled in a way

analogous to implantable drug delivery systems whereby the septa is penetrated by a syringe needle through the skin.

The sensing system in accordance with the invention results in dramatic improvement in the stability of the thermopile, and even other types of amperometric biochemical sensors, resulting from the constant renewal of the chemical reagent system. The reagent is not limited to a fixed amount that is typically trapped behind a membrane, for example, as in the case with conventional enzymatic sensors. Enzymes and other organic chemical systems typically have longer shelf life compared to operational life, e.g., when in continuous contact with an environment having the target-chemical. Changes in the activity of the stored reagent over time typically do not have substantial effect on the senor operation. The period of reagent stability, of course, varies depending on the reagent system.

In another embodiment the thermopile **80** sensor which is illustrated by Fig. 1 and Fig. 3, has several electrical contacts **110** and **111** which tap the series of thermoelectric junctions at fixed intervals. These taps are useful in combination with a hollow membrane microflow system whereby the enzyme moves progressively over the duration of its forward progress to a different set of thermoelectric junctions. The process is one whereby the new entering enzyme generates a greater heat for the thermoelectric junctions **110** more proximal to the new enzyme while the older and more decayed enzyme generates a lesser heat over the more distal thermoelectric junctions **111** along the flow stream. By a process of comparing the thermoelectric signal **120** from the proximal sets of junctions which are in contact with the new entering enzyme, to the distal thermoelectric junctions whose signal **121** corresponds to that of an older and therefore more decayed activity, it is possible for the microcontroller 130 to estimate and thereby compensate for the rate of enzyme decay.

In a further non-limiting embodiment, a method is provided for recalibration whereby the electrical signals from subsets of thermoelectric

junctions are measured by an automated electrical or computer based system **130** so as to compare the relative activity of the enzyme just entering the membrane fiber at one end of the thermopile sets of junctions **110** to that at the other end **111** where the enzyme exits. The difference between these two signals can be used to estimate any changes in enzymes rate of decay in-situ and to effect a continual recalibration of the sensor.

In an alternate sensing arrangement, the present invention includes (i) an optical cell and (ii) microdialysis tubing. This sensing arrangement combines microdialysis sampling techniques with the use of a microflow system employing an optical cell to create a system that can accurately measure the concentration of glucose and other chemicals in complex solutions bearing proteins. This particular approach has advantages which allow its use in diverse applications, such as for cell culture biochemical measurement, as well as for implantable applications for blood glucose measurement.

In this embodiment, the biochemical sensing system, as illustrated by Fig. 5, includes a pressurized container **300** which includes collapsible bags, typically made of MYLAR, **304** for holding reagents, calibration solution **302**, and sweep solution **306**. These are regulated in their flow by resistance tubing, as hereinbefore described, whose diameter and length can been selected to achieve flow rates typically in the sub-microliter per minute regime. For example, a 25 centimeter length of 15 micron diameter silica resistance tubing, and with a 10 psi charging pressure in the container squeezing on the reagent bag will produce a flow rate of approximately 300 nl/minute.

The sweep solution is typically regulated to a flow rate which is slower by about 20-50 times, i.e., in the tens of nanoliter per minute rate, than the reagent in order to achieve a correct proportion in mixing. Although the mixing ratios will differ according to a specific reagent, the mixing ratio reagent to sweep fluid for the Trinder test is from about 20:1 to about 200:1. The sweep solution is introduced by connecting tubing, typically microbore tubing, to a microdialysis

fiber **308** that is in diffusive contact with the test environment **310**, e.g., a bioreactor perfusion loop. At flow rates of approximately 300 nl/min. and a retention time of about 2 minutes through a microdialysis fiber **308** of about 10 to 30 mm. long, the target-chemical concentration in the sweep fluid can reach diffusive equilibrium with the test environment. The return dialysate (i.e., sweep fluid containing the target-chemical) is then mixed with the particular reagent. The mixed solutions move down a single tube or capillary **330** where the chemical reaction of the reagent with glucose proceeds and the optical change occurs, i.e., the reagent-dialysate mixing volume. The absorbance of the flow stream at the specific color of a chemically sensitive dye is measured by an optical cell **320** having a light emitting diode and miniature diode photodetector. The resulting photodetector signal is calibrated in terms of glucose concentration by the microcontroller **340**.

The microdialysis tubing **308**, also referred to as a membrane hollow fiber, in contact with the test solution test is made from a material which is permeable to glucose but excludes large molecular weight materials. Typically, the microdialysis tubing is made of materials such as cellulose acetate, polysulfone, and polyacrylonitrile, usually in the form of hollow tubes on the order of 200 microns in diameter. The reagents that are mixed with the sweep fluid are chosen so that their color or fluorescence change has a specific response to the biochemical desired, as is well known in the art.

An optical cell **320** at the receiving end of the mixed reagent flow stream measures color or fluorescence change, and the signal obtained therefrom is related to chemical concentration by microcontroller **340**. A suitable optical cell is shown in Figure 7. The optical cell includes a light transparent capillary tube **462** having a light emitting diode **464** and photodiode **466** placed on opposite sides outside of the capillary tube **462**. The optical cell **320**, constructed of capillary tubing approximately 100 microns in diameter, mates directly to a Y-connector where mixing of the dialysate and the reagent occurs **330**. For example, in the case of a

Trinder based absorbance glucose sensor, blue excitation light from a high intensity light emitting diode (LED 505 nm for Trinder test) passes through the developed dye in the optical flow cell. The photodiode has a light approximately 0.5 mm square and is mounted directly against the capillary tubing. The total volume of the developed reagent contributing to the detected absorbance signal is approximately 300-600 nl.

The electronics support system for the optical fluorescence cell includes an oscillator-driver which applies a square wave current to the blue LED for dye fluorescence excitation. The absorbance is detected by the photodiode and is demodulated to determine its amplitude by a lock-in amplifier.

The test environment may be remote from the reagent reservoirs and the microdialysis sampling fiber connected to it by microbore tubing. The diameter of the interconnecting tubing is preferably exceedingly small, typically 35 to 44 gauge, and short as possible so as to minimize the hold up volume and reduce its loop transit time back to the waste reservoir.

The optical cell volume of the capillary is typically on the order of about 100 nanoliters to about 1 micro liter. An optimum mixing ratio can be experimentally determined by taking into account the trade-off between linearity at high dilutions versus reduced reagent consumption rate at low flow rates. The mixing ratio of reagent to dialysate is determined by the respective flow rates of the dialysate and a particular reagent.

In an alternate embodiment of this sensing arrangement, a Trinder reagent test employing the enzyme glucose oxidase is used to give a specific colorimetric response to glucose in the microdialyzed flow. The mixing ratio of reagent flow to sweep flow is typically about 40:1. In another embodiment, a toluidine based reagent for glucose is used and after mixing with the sweep fluid, is heated by passing it through a capillary tube heated by a resistance wire to 100°C required for color development. The mixing ratio of toulidine flow to sweep flow is typically about 5:1.

This invention can also advantageously employ multiple reagents for multiple analytes in combination with multiple optical cells which measure different optical properties of the mixed reagent system. Each property (e.g., absorbance or fluorescence) can be measured independently or simultaneously. Alternately, the reagents added to the incoming dialysate can alternate to serially determine the presence of multiple substances in the dialysate. In these embodiments, microvalves **360** can be used to turn on or turn off the flow rate of any of the reagents.

The colorimetric test is a robust approach to measuring chemical concentration and does not exhibit the problems of electrical drift that are common with electrochemical techniques. For example, amperometric electrodes experience slow changes in their platinum electrodes, enzymes, and silver reference electrodes. Like electrode type sensors, the reagents for the optical cell typically have a longer shelf life than if they were in continuous contact with an environment containing the target-chemical. These chemical tests reach an endpoint where the absorbance reaches a final value. If enough time is allotted for development, the enzyme activity and its corresponding rate of development does not affect the accuracy of the measurement. In contrast, most other sensors depend on the rate (i.e., activity) of the enzymatic reaction to provide an accurate reading. There is little else about the system that is subject to aging effects.

In one embodiment, this sensor approach continuously mixes fresh reagent from the supply reservoir with the microdialysis sample stream. In another embodiment, the flow streams can be shut off by the employment of microvalves **360**. This can be advantageous where continuous monitoring is not required and it is desired to prolong the lifetime of the sensor reagent supply. In either embodiment, the mixture is carried by its flow past the optical cell **320**.

The sensor system has a potentially long lifetime because (i) a relatively large amount of the optical reagents can be stored in a remote reservoir and (ii) slow flow rates are used. In comparison, most conventional electrodes contain

fixed amounts of the optical reagents. Typical flow rates of the sweep fluid are on the order of 10-50 nl/min while the flow rate of the reagent is on the order of 100 nl to 1 microliter per minute. These flow rates allow month-order and longer continuous monitoring sensor operation with reagent reservoir volumes less than 25 milliliters.

The sensing arrangement according to the invention may be implanted to provide glucose sensing by introducing the microdialysis interface, as a catheter, to the portion of the body where it is desired to measure glucose concentration. This might be in the lumen of the heart, to a blood vessel using a suitable interface, or to the peritoneal cavity. The reagent supply and optical readout would typically be implanted in a way similar to that of a pacemaker, as shown in Fig. 4.

The reagent supply reservoir and sweep fluid reservoir must be replenished periodically, typically by methods similar to the replenishment of insulin in implantable insulin delivery pumps. This is performed by the use of a coaxial syringe needle puncturing a septum on the reagent supply casing and refilling the distensible supply bag while using the second needle lumen to remove waste in accordance with the arrangement shown in Fig. 4. Catheter style interfaces of the microdialyis fiber consist of integrating the microdialysis interface into the distal and surface of a catheter. Interconnection to the microdialysis fiber is by way of microbore tubing running through the catheter. In this configuration, the sensor system for glucose resembles that of a pacemaker, and indeed might be implanted in a similar way to sample the glucose concentration in the blood of the heart via a catheter introduced into the heart in the same way as a pacemaker lead.

In one embodiment, sampling of media is by way of a catheter made from silicone rubber containing a window exposing the membrane hollow fiber to the solution. The sweep fluid is circulated by flow through the catheter loop and returns to mix with the reagent flow stream and than pass on to the optical cell. Following the optical cell is another collapsible waste reservoir **350** that holds the developed reagent as a waste product that ultimately will be retrieved.

In another embodiment, the microdialysis fiber is in direct contact with a flow stream of a cell culture media by employing a flow interface. This interface need not be much more than a loop-through of the microdialysis fiber through the walls of a bioreactor or with a portion of the flow loop tubing such that the fiber comes into contact with the media.

Colored calibration fluids **302** which are insensitive to the target-chemical concentration (e.g., insensitive to glucose concentration) yet have known optical properties can be introduced into the loop periodically to verify the operation of the flow loop and optical cell operation.

The present sensing system also provides an arrangement for detecting a gas which includes (i) a optical cell and (ii) gas-permeable microdialysis tubing. This embodiment combines optical principles for measuring oxygen and/or carbon dioxide in conjunction with a microflow system that circulates a gas sensitive reagent through a gas-permeable membrane hollow fiber that is placed in contact with a test medium. The sensors described in Towe et al., "A recirculating-flow fluorescent oxygen sensor," Biosensors & Bioelectronics, Vol. 11, No. 8, pp. 799-803 (1996), which is incorporated herein by reference, can used according to the present embodiment.

As shown in Fig. 6 gas sensitive reagents are circulated through microbore tubing **430** up the length of a catheter **435** to a gas-permeable hollow membrane fiber **440** at its end which is exposed to the blood or solution under test **450**. The membrane hollow fiber is made from silicone or other gas permeable material which excludes diffusion therethrough of dissolved non-gas substances, thereby preventing contact of the non-gas substances with the reagent. The returning reagent flow stream is in equilibrium with the gas concentration at the sensor tip and affects the optical properties of the reagent. The reagents circulated are chosen so that their color or fluorescence change in specific response to the gas desired. A micro-optical cell **460** at the receiving end of the circulating reagent flow stream

measures the color or fluorescence change, and the signal derived from the optical cell is related to chemical concentration by a microprocessor.

Silicone tubing, being relatively permeable to gases (i.e., oxygen and especially carbon dioxide) yet hydrophobic, can effectively entrap liquid solutions of reagents and gas-sensitive dyes while preventing their diffusion into the test media, e.g., a blood flow stream. In addition, a single loop of the silicone tubing can alternately measure either oxygen or carbon dioxide while *in situ* without replacing the catheter or need for a second flow loop by alternately infusing the corresponding optically responsive reagent in the tubing loop.

This kind of sensor strategy permits a longer dye lifetime than fiber optic approaches since (i) the optical reagents are constantly renewed at a relatively low flow rate and (ii) the relatively large amount of the dye can be stored in a remote reservoir. As a result, small amounts of the optical reagents which may leach out from the tubing can be replaced by the flow. Also the optical signal derived from the reagent is relatively larger compared to that obtained, for example, from fiber optic systems because of the larger volume of fluid, as much as ten times, being illuminated at the optical cell. This typically results in good signal to noise ratios. Additionally, the sensing reagent is subjected to a lower intensity of light for fluorescence or absorbance tests because of the relatively higher signal level. Hence problems with reagent bleaching due to continuous exposure to intense light do not affect the present sensor operation to the extent of the prior art sensors.

Colored calibration fluids, not shown in Fig. 6, which are insensitive to the target gas concentration yet have known optical properties can be introduced into the loop periodically to verify the operation of the flow loop and optical cell operation.

For example, oxygen concentration can be determined by measuring fluorescence of an oxygen quenched dye exiting a thin silicone tube which is immersed in the test solution. The flow rate of the dye is slow enough so that the oxygen concentration in the dye solution achieves diffusive equilibrium across the

silicone membrane with the dissolved oxygen in the test solution. A preferred silicone tubing is #508-001 available from VWR Scientific Products, Salt Lake City, Utah, and typically has an outer diameter of about 1.65 mm, an inner diameter of about 0.76 mm, and a length of about 0.5-1.0 cm. The flow rate of the dye through the tubing is typically in the range from about 300 nl/min to about 1 µl/min. Partial pressure equilibrium of oxygen occurs through the wall of the tubing in residence times of a minute or so as a result of the high permeability 50 x 10^{-9} cm^3/sec cm^2 cm-Hg of silicone for oxygen as described in the product information for Zero Corp, located in North Salt Lake, Utah (1993). The portion of the dye exiting from an in-line optical cell is returned to a waste reservoir.

As illustrated in Fig. 6, the outlet flow of a pressurized reservoir of fluorescent reagent **410** is regulated by employing a length of 15-25 µm silica resistance tubing **420** having an inner diameter of about 15-25 nm. By applying positive pressure via an unreactive gas to a collapsible bag and selecting the resistance tubing diameter and length, steady flow rates on the order of about 10 nl/min to about several microliters per minute through the fluid loop can be readily achieved. Such flow rates reduce the consumption rate of the dye to levels where the lifetime of the sensor can be extended to month-long periods using only a several milliliter supply of reagent. With the dispensing of all the reagent, the sensor can be either refilled or a new cartridge can be connected to the sensor.

As illustrated in Fig. 7, the optical fluorescence cell **460** is constructed of glass, TEFLON, or quartz capillary tubing **462** approximately 100 microns in diameter and mates directly to the silicone tubing entering into the sensor body. Blue excitation light from a high intensity blue light emitting diode (LED) **464** passes through the dye in the optical flow cell. It is typically blocked from direct illumination of the photodetector by a broad bandpass red celluloid filter, not shown, that transmits the dye fluorescence at 620 nm and blocks the blue excitation source. The photodiode sensitive area **466** is approximately 0.5 mm

square and is mounted directly against the capillary. The total volume of the dye contributing to the detected fluorescence signal is approximately 0.5 µl.

The electronics support system for the optical fluorescence cell consists of an oscillator-driver which applies a 400 Hz square wave current to the blue LED **464** for dye fluorescence excitation. The time varying fluorescence is detected by the photodiode **466** and is demodulated to determine its amplitude by a lock-in amplifier.

By way of example, the oxygen-quenching fluorescent dye ruthenium tris-(2,2'-bipyridyl) II dichloride (RTDP, formula weight 640.5) is used in the present gas sensors. It's use was reported in Wolfbeis, et al., "Fiber Optic Flurosensor for Oxygen and Carbon Dioxide," Anal. Chem., 60:2018 (1988) and Wolfbeis et al., "Recent Progress in Optical Sensor Design," Proc. SPIE, 906:42 (1988). Other fluorescent dyes sensitive to oxygen are known to the art and can be used herein.

In a suitable arrangement the oxygen sensor uses an oxygen-sensitive fluorophore, e.g., tris (2,2,'-bipyridine)-ruthenium(II) chloride perfused at 10 µl/min. through a 14 cm silicone hollow fiber membrane (0.305 mm i.d. x 0.508 mm. o.d.). A 470 nm LED, available from Panasonic LNG992CFBW, excites the 463 nm excitation peak of the fluorophore through a 0.635 mm translucent capillary. The 620 nm peak is monitored with an orange coated (Orange Poppy transparent glass paint available from Plaid Enterprises Inc.) photodetector which is available from Texas Instruments TSL 235.

In a preferred embodiment, a catheter made from a gas impermeable tubing connects to a gas permeable segment in a region where the gas concentration is to be measured. The dye is circulated by flow through the catheter loop and returns to pass through the optical cell. The two types of tubing can be interconnected using any means known in the art, such as via a short length of stainless steel tubing, e.g., syringe needle, placed within the lumens of the two tubing, or via a coaxial press fit of two different sized tubings, typically secured by a silicone glue.

Measurement of a gas such as carbon dioxide tension may advantageously be performed by way of a phenol red dye in a bicarbonate buffer solution which is circulated through the hollow membrane fiber at the end of the catheter. This solution changes color specifically in response to carbon dioxide concentration and its properties and composition are well known to the art. In this embodiment, the returning flow from the catheter tip is subjected to a light of a 535 nm wavelength, typically generated by a light emitting diode; its absorbance at this wavelength is measured by a photodiode; and the resulting signal is calibrated to carbon dioxide concentration.

The silicone tubing is particularly permeable to carbon dioxide, and so is a suitable hollow membrane fiber. It allows the reagent solution to respond selectively to carbon dioxide without interference from the pH of the test solution or other substances in the environment.

The response time of this sensing arrangement is determined primarily by the flow rate of the dye and the length and hold up volume of the tubing in the return flow loop. For this reason, it is desirable to use very small diameter fluid-return tubing, such as number 36-40 gauge tubing to minimize this delay.

The reproducibility of this sensor is excellent; generally maintaining within +/- 1% of its initial reading after exposure to a transient change in oxygen tension. No measurement hysteresis, or unaccountable baseline shift is typically observed, and the reagents do not exhibit time-dependent changes in their sensitivity or other characteristics.

Flow-type sensors transport microquantities of oxygen by diffusion through the silicone tubing wall to or from the immersion solution. These small quantities can affect the sensor performance by creating an oxygen boundary layer build-up phenomena around the silicone tubing and changing the oxygen concentration in the local microenvironment next to the tubing. Without wanting to be limited by any one theory, it is believed that the sealed feed reservoir of fluorophore circulating solution brings with it a potentially different concentration of oxygen

than in the test solution. As a result, stagnant solution creates measurement errors if the solution convection is not adequate to disperse this build-up. It has been found that small diameter silicone diffusion tubes and low dye flow rates, typically less than 1 µl/min. transport less oxygen to the test solution and effectively solve this problem in all but stagnant test solutions. In stagnant test solutions, the flow rate is preferably below about 0.5 µl/min.

In another embodiment, a sensing arrangement according to the invention includes a glucose sensor that employs a commercial e-PTFE vascular graft modified to incorporate a microdialysis fiber as an interface to the blood stream. The low flow rate of a sweep fluid from the sweep fluid reservoir allows the target chemical to enter the sweep fluid via diffusion from the blood stream through the e-PTFE vascular graft and the microdialysis fiber. The dialysate containing the target chemical at the concentration of the blood stream emerges from the fiber and continuously mixes with another microflow stream containing a colorimetric reagent. Glucose concentration is subsequently monitored by absorbance changes in the mixed flow by using a small optical absorbance cell having a light emitting diode and a photo detector. This approach takes advantage of the biocompatibility of vascular grafts as a means of interfacing sensors to the blood stream. It also employs the notion of a continuous-flowing microfluidic type of glucose sensor. *In vitro* evaluation of the sensor system in blood has shown that the system is accurate to within +/- 12 mg-% in 16 minute response times.

The specific embodiments of a sensing arrangement and methods of detection of a target chemical in accordance with the present invention will now be described in further detail. These examples are intended to be illustrative and the invention is not limited to the specific materials and methods set forth in these embodiments.

Example 1

Implantable Microfluidic Glucose Sensor

An implantable microfluidic glucose sensor **500** is shown in Fig. 8. This pacemaker-like device includes a containment **550** having a compartment housing a sweep fluid reservoir **554** and a reagent reservoir **556** connected by microbore tubing **558** to the microdialysis tubing **520** which is fixedly attached and in diffusive contact with a e-PTFE sheath **510**. The e-PTFE sheath is in fluid contact with the sample test media. The optical cell **540** includes a capillary tube having a light emitting diode (LED) **542** on one side and a photodetector **544** on the other side is housed in a second compartment which incudes telemetry equipment. A waste fluid reservoir **560** is housed at a lower pressure than the compartment housing the sweep fluid and the reagent. The waste fluid reservoir is typically placed outside the containment which is pressured with perfluorocarbon propellant **552**. The waste fluid reservoir **560** can also be covered by an expandable biocompatible material **570**.

Example 2

Sensing Arrangement Including Vascular Interface

A sensing arrangement as shown in Fig. 9 including a vascular interface **600** was used to measure glucose concentration. The sensing arrangement also included a syringe pump **610**, a development tube **620**, a roller pump **630**, reservoir **640**, an optical cell **650**, a microcontroller **660** and a personal computer **670**. The vascular interface **600** includes dialysis fiber **602** within the lumen of a e-PTFE tube **604**. The development tube **620** is 0.065" (1.65 mm) in diameter and approximately 10 cm long. The Trinder reagent flows from a first syringe **612** directly into the development tube **620** via microbore tubing. The sweep fluid coming from a second syringe **611**, also driven by the syringe pump, passes by way of microbore tubing to the microdialysis fiber **602** embedded within the vascular graft interface **600**. The dialysis fiber exit is mated to a microbore

polyimide tube that carries the dialysate to the development tube **620**. The two flow streams meet at the entrance of the development tube and proceed to mix together as they proceed by flow to progress down the tube. The optical cell 650 is placed so that it views the flow stream at 4.1 cm downstream from the point where the Trinder reagent and the diasylate sweep fluid meet. The flow transit time to arrive at the optical cell provides time for the chemical reaction of the Trinder reagent and sweep fluid to fully develop and achieve maximal absorbance.

The microdialysis fiber was prepared as follows. A regenerated cellulose (RC) fiber was extracted from a Terumo Clirans 220 hemodialyzer manufactured by the cuproammonium rayon method. The inner diameter of the dialysis fiber was 200 μm, the wall thickness was 9 μm, and the molecular weight cutoff (i.e., for a target chemical able to diffuse therethrough) was 35,000 Daltons. The fibers were prepared by first removing a single dialysis fiber from the dialyzer, cropping the fiber to a length of 6 cm and then inserting a 100 μm platinum wire into the fiber lumen to allow easier handling and to add structural support.

The system of polyimide microbore tubings, available from HV Technologies located in Trenton, GA, used to conduct flows to and from the microdialysis fibers play a critical role in determining sensor response time characteristics. Approximately 30 cm of #44 kink resistant polyimide tubing (51μm I.D.) was attached to the dialysis fiber by silicone adhesive. Similarly, 15 cm of #37 kink resistant polyimide tubing (114 μm I.D.) was connected to the exit of the dialysis fiber. The use of the larger inner diameter tubing at the exit of the fiber ensures that a build-up of pressure doesn't occur within the dialysis fiber, forcing water across the membrane wall and affecting the measurement accuracy.

Expanded polytetrafluoroethylene (e-PTFE) vascular grafts of 4 mm inner diameter were donated from Atrium Medical Corporation of Hudson, New Hampshire. Microdialysis fibers were integrated into the grafts in several configurations. In one configuration tested shown in Fig. 10A, a 200 μm o.d. dialysis fiber **700** was passed at a shallow angle through the e-PTFE graft wall **702**

such that it was parallel with the inner lumen. The dialysis fiber was secured to the e-PTFE using a silicone adhesive **705**. The dialysate inlet **706** and outlet **708** are also shown. Approximately 1.5 cm of its length was exposed to the test solution flowing through the graft. In another configuration shown in Fig. 10B, the dialysis fiber **700** was placed within the thickness of the e-PTFE wall of the graft **702**. This was accomplished by thinning down a portion of the graft wall with a sharp razor blade so that a layer (0.5mm) of the wall remained. The slit was approximately 3.5 cm long and 0.5 cm wide. A microdialysis fiber was placed on the cut down region and a 1 x 4 cm piece of polyethylene film **704** was used to seal in the fiber and add support. A cyano-acrylate glue was used to secure the polyethylene film.

A variation of this arrangement involved additionally perforating the thinned graft wall with a 25-gauge hypodermic needle to enhance diffusion. Approximately 25 punctures where evenly spaced over the 3.5cm length. This enhanced filtration of fluid from the lumen. This configuration was used to maximize transport and test the response of other parts of the sensor system.

This sensor arrangement flows distilled water through the microdialysis fiber. Glucose diffuses or dialyzes across the membrane from the bulk media. As shown in Fig. 9, the dialysate then flows into a larger diameter and several centimeter long developer tube **620** where there is added a flow of glucose-sensitive Trinder reagent available from Sigma Inc. Mo. The colorimetric absorbance is measured as the flow moves into a glass capillary acting as an optical cell **650**.

Flow of the dialysate was at 263 nl/min and the Trinder reagent at 10μL/min. The length of the development tube was chosen such that there was a delay of approximately 10 minutes for the dye to develop prior to reaching the optical cell. The dye absorbance was measured by passing the development tube between a 505 nm LED (LEDtronics Inc, USA) opposite a pre-amplified photodetector (OPT 201, Burr-Brown, Tucson, AZ). The amount of light that reaches the photodetector is a function of the sample absorbance. The volume of

fluid exposed to the light within the optical cell was 122nL. Low pass filtering of the absorbance signal was achieved by averaging the measurements over four minutes.

Example 3

Calibration of Sensing System

The sensing system of Example 2 was tested and calibrated with glucose buffer standards flowing through the vascular interface at 30 ml/min. In the first configuration, where the fiber was located along the wall of the graft, calculations show that sweep fluid residence times of thirty seconds resulted in a 90% equilibration with the surrounding bulk flow. In the configuration where the fiber was integrated into the perforated wall of the vascular graft, the residence time needed to achieve 90% equilibrium extended to 220 seconds. Glucose diffusion through thinned e-PTFE walls but not perforated did not achieve equilibrium with the sweep flow in any reasonable residence time tested. Manual methods were not successful in thinning the wall much more than 500 microns. It may be that other types of graft materials are more permeable.

The effect of different mixing ratios of the dialysis sweep fluid flow to the reagent system flow can be used to determine the optimum mixing ratio. Lower dilution ratios result in a desirably higher sensitivity to glucose but result in reduced range of linear response. Lowered ratios were also found to make the system sensitive to irregularities in flow rates and proportioning errors from syringe pumps that were operated below their rated flow rates. Ten-minute development times resulted in a stable color change that translated to a glucose uncertainty of ±5mg/dL. The 38:1 dilution ratio used allows for a linear range up to 300mg/dL.

Whole beef blood was used to test an integrated sensing system in accordance with Figure 9. More particularly, the sensing system's response to step changes in glucose concentration was investigated. The results of these tests are

shown in Fig. 11. The glucose concentration of the bovine blood was increased by stepwise addition of powdered glucose to the flow loop. The decrease in glucose concentration was caused by replacing the circulating blood with fresh blood that had the original concentration. The average response time was 16 minutes, consisting of approximately a 12-minute pure delay due to various fluidic hold up volumes and a rise time of 4 minutes and a decay time of 5 minutes. Fig. 11 also demonstrates the glucose sensor system is quite accurate, i.e., +/- 5%, and is able to measure glucose concentration in a complex solution like bovine blood.

Example 4

Determination of Optimum Flow Rate In Graft Sensing Systems

Microdialysis sensors consume their substrate. Glucose in the local environment diffuses through the membrane fiber and is transported away from the site by flow. As a result, the rate of diffusion of glucose to the sensor site must be faster than its removal by the dialysis fiber to allow the sensor to reflect the concentration of the test solution. Additionally, the sensor system depends on the emerging sweep fluid concentration to be in essential equilibrium with the surrounding glucose value. This means that the sweep fluid must flow through the fiber slow enough to achieve a relatively high extraction fraction (E), which is the ratio of target chemical concentration in the dialysate and the target chemical concentration in the test medium, given by the following equation:

$$E = 1 - \exp\left(-\frac{1}{QR}\right)$$

where Q is the flow rate of the dialysate and R is the resistance to analyte diffusion into fiber. This resistance in turn depends on not only the membrane hollow fiber resistance, but also that of any overlying layer of PTFE, as well as on the fluid convective environment around the fiber.

When the membrane fiber is placed in the lumen of an e-PTFE graft the sensing system performs adequately and at a given sweep flow rate the extraction

is high. However, the diffusion resistance of 0.5 mm of e-PTFE of the type used is too high to support the rate of glucose removal by dialysis, at least at sweep flow rates of 263 nanoliter per minute. Much lower sweep fluid flow rates may help address this problem.

Perforation of the thinned graft wall using small holes to reduce diffusion resistance resulted in reasonable response times but is not likely to be useful in practice. Perforation compromises the integrity of the graft and act as sites of thrombosis and would seal off quickly *in-vivo*. Integration of the fiber into the polymer matrix of the graft so that it resides within a few tens of microns of the wall should substantially reduce resistance and can be a more mechanically robust approach. It was found that the lowered mechanical strength of thinned wall vascular grafts are not sufficiently stable under arterial pressures as great as 200 mmHg.

Fig. 12 illustrates residence times using alternately a regenerated cellulose (RC) microdialysis fiber or AN69 microdialysis fiber when specifically placed within a perforated pocket and within 0.5 mm of the inside wall of the e-PTFE vascular graft in accordance with Example 2. This plot illustrates the slowing down of the equilibration process due to the increased resistance of the e-PTFE to diffusion of glucose therethrough.

Example 5

Determination of Optimum Ratios

A fixed quantity (2 ml) of commercially available Trinder reagent, prepared according to the manufacturers directions was placed in a glass cuvette. Variable amounts of glucose were added. Absorbances were measured by a spectrometer. More specifically, at each known concentration of glucose (0, 100, 200, 300, and 500 mg-%) there were four different volumes 100 nl, 40 nl, and 10 nl added in separate trials to the Trinder reagent, thereby achieving mixing ratios of 20:1, 50:1, 100:1, 200:1.

The Trinder reagent darkens with increasing concentration of glucose added. Fig. 13 shows that at high dilution ratios (e.g., 200:1) the sensitivity is relatively low since the changes in absorbance with glucose concentration are relatively small. At lower dilution ratios, the sensitivity is higher but the readings at different concentration suffer from nonlinearity resulting from the smaller change in absorbance at higher concentrations of glucose. The actual dilution ratio used was 38:1 which was a compromise between sensitivity and linearity.

Example 6

Determination of Optimum Residence Time

An experiment directed to determining the fraction of glucose equilibrium to the sampled solution versus the residence time of a saline sweep fluid in a cellulose acetate hollow membrane fiber was performed as follows.

A 2 cm long loop of 200 micron i.d. (628 nl total volume) Cuprophane dialysis fiber available from Enka Inc. was introduced into a coaxial countercurrent circulating flow stream of 100 mg-% glucose solution pumped at a velocity of 2.1 cm/second past the fiber. A second syringe pump was used to pass a water sweep fluid through the dialysis fiber at various flow rates in the range of approximately 238 nl/min to 2.3 nl min.

After passing through the fiber, the emerging slow flow of dialysate was collected and spectrophotometrically analyzed for glucose concentration by standard laboratory techniques. The ratio of the measured glucose concentration in the dialysate to the actual concentration in the circulating flow stream is expressed as a fraction of equilibrium.

A plot of these ratios is shown in Fig. 14. The graph shows, for the specific Cuprophane membrane shown, that the sweep fluid within the fiber achieves greater than 95% diffusive equilibrium with the glucose concentration in the flow stream in times equal to or greater than 150 seconds. This experimental result defines the flow rate through the microdialysis fiber needed to achieve adequate

sampling of the circulating glucose solution. In practice, the membrane fiber is brought into contact with blood, cell culture broth, or other media or material containing glucose that is diffusible across the membrane fiber whose concentration is to be monitored.

Example 7

CO_2 Sensing Arrangement

This sensing arrangement used a 5 cm length of small diameter silicone tubing (0.305 mm i.d. x 0.61 mm o.d., Dow Corning Inc.) through which a sweep fluid was passed at 3.8 nl/min. The sweep fluid consisted of a 0.04 wt% phenol red in a 35 mM bicarbonate buffer. This solution is known to the art as a concentration indicator and has the property of changing its absorbance in proportion to the dissolved CO_2 over a range of concentrations that are of interest in biological and living systems. The silicone tubing with the indicator sweep fluid acts as the primary sensor element. The sensor system response to CO_2 was measured by placing it in a coaxial countercurrent flow stream of water moving past it at a velocity of approximately 3 cm/sec. The CO_2 concentration of this flow stream was adjusted by an external system employing a membrane gas device that is well known to those in the art.

The residence time for the sweep fluid in the silicone tubing was 1.05 minutes. Silicone tubing is highly permeable to CO_2, and it was found that residence times greater than approximately 30 seconds were adequate with the employed silicone tubing to ensure greater than 95% CO_2 diffusive equilibrium with the surrounding flow stream. The approximately one minute residence time actually employed was used as an extra margin of safety to insure nearly total CO_2 equilibrium with the surrounding flow stream. This was also done to minimize changes in the system calibration as the silicone tube becomes coated with contaminants carried by the measured flow stream that may tend to adhere to the tubing and reduce CO_2 diffusion across the membrane.

The optical absorbance of the emerging sweep fluid was measured by flowing it through a 0.635 mm diameter glass capillary tube and placing a 555 nm (green) light emitting diode and a standard photodetector diode (Burr Brown OPT-201) across the flow stream. Variations in the absorbance of the sweep fluid are proportional to CO_2 concentration and these cause a greater or lesser amount of light to fall on the photodetector. The output voltage of the photodetector is thereby proportional to the CO_2 concentration. This electrical voltage is subsequently signal processed by a microcomputer system to linearize the response. There follows a conversion of the voltage to a chemical concentration value through a computer based calibration look-up table as is common in the art. A plot of the output voltage versus CO_2 pressure is shown in Fig. 15.

Example 8

Determination of Optimum Glucose Response Range

A microdialysis fiber was constructed by introducing a 3.5 cm long loop of 200 micron i.d. (628 nl total volume) Cuprophane dialysis fiber available from Enka, Inc. into a coaxial countercurrent circulating flow stream of buffer test solution pumped at a flow velocity of 2.1 cm/second past the fiber. The glucose concentration of the solution was an experimental variable and adjusted by adding glucose to the circulating solution in quantities sufficient to achieve the required concentration increments shown. A pump was used to pass a water sweep fluid through the dialysis fiber at a flow rate of 263 nl/min such that the residence time of the sweep flow within the fiber was 4.1 minutes. As shown in Fig. 14 this resident time was found sufficient to achieve greater than 98% diffusive glucose equilibrium with the surrounding flow stream.

The distal end of the dialysis fiber together with a small polyimide tube (114 nm i.d.) was epoxy glued into a 1.6 mm diameter glass capillary tube. A separate polyimide tube conducted a flow stream of Trinder reagent available from Sigma Chemical Co. at a flow rate of 10 nl/min into the glass capillary. The exit

ends of both tubes were aligned so that both flow streams entered the capillary at the same point. These two flowing solutions, the dialysate and the Trinder reagent, mixed together as they progressed down the length of the capillary tube. The mixing ratio of these two streams is determined by their relative flow rates. In this example, a mixing ratio of 38:1 was chosen. This dilution ratio was selected as a compromise between linearity of response with increasing glucose concentration, sensitivity, and consumption rate of the reagent. Higher dilution ratios, up to 200:1 are recommended by the manufacturer to maximize range of linear absorbance change with concentration. Lower dilution ratios cause a greater absorbance change for a given concentration change and thus increase sensitivity. Lower dilution ratios also reduce the flow rate of the Trinder in proportion to the sweep flow and so desirably reduce the consumption rate and storage volumes of the Trinder. In this example, it was desired to increase sensitivity and reagent consumption at the expense of linearity since the glucose sensor was intended to operate over a fairly narrow physiologic range, extending from 0 m-% glucose to as high as 200 mg-% glucose, and not as high as 2000 mg-% as a 200:1 dilution ratio would afford.

The optical cell was placed 4.6 cm downstream of the mixing point of the reagent and dialysate such that the transit time to the cell equaled about ten minutes. This interval gave sufficient time for intermixing and a chemical reaction process to occur that resulted in the darkening of the Trinder reagent in accordance with the manufacturer's development time specification.

The optical absorbance of the flow stream was measured by placing a 505 nm (blue-green) light emitting diode and a standard photodetector diode (Burr Brown OPT-201) across the photodetector such that the flow stream intercepted the light path. This color was chosen to match the color of maximal absorbance of the Trinder indicator. After passing the optical cell, the flow stream continues on to a waste container.

The photodetector was arranged and baffled such that only transmitted light through the flow stream fell on its photosensitive surface. A variation in the absorbance of the flow stream is proportional to glucose concentration and this causes a greater or lesser amount of light to fall on the photodetector. The output voltage of the photodetector is thereby proportional to the glucose concentration. This electrical voltage is subsequently signal processed by a microcomputer system to linearize the response. There follows a conversion of the voltage to a chemical concentration value through a computer based calibration look-up table as is common in the art. A plot of the output voltage versus glucose concentration is shown in Fig. 16.

The sensing system has a response time which can be reduced by adjusting a number of variables. The 30 second and 220-second sweep fluid residence times are defined by the convective environments and are relatively fixed. The typically ten-minute development time for the reagent is defined by the use of Trinder reagent. Additions of glucose oxidase to this standard test can reduce the development time to less than five minutes. The lengths of the interconnecting tubing creating hold-up volume delays are the remaining factors contributing to the overall approximately 16 minute response time. With some effort these contributions might be reduced to near zero. This would result in a sensing arrangement whose response time is the sum of the development time and the sweep fluid residence time; perhaps on the order of five to seven minutes.

Although the invention has been described herein with reference to specific embodiments, many modifications and variations therein will readily occur to those skilled in the art. Accordingly, all such variations and modifications are included within the intended scope of the invention.

WHAT IS CLAIMED IS:

1. A sensing system for determining the presence of a target chemical in a test fluid comprising:

a micro-flow reservoir system having at least one micro-flow reservoir including a reagent fluid comprising a sensing substance which reacts with the target chemical,

a sensor system comprising a thermopile for detecting the occurrence of said reaction connected to the micro-flow reservoir system, and

a conduit connecting the micro-flow reservoir system and the sensor system for conveying reagent fluid in the micro-flow reservoir system to the sensor system,

wherein the sensing system is capable of being immersed within said test fluid.

2. A sensing system according to claim 1 wherein the sensor system includes:

a thin film thermopile sensor having a plurality of sensing junctions and a plurality of reference junctions;

a hollow membrane fiber disposed proximate to each of said sensing junctions, wherein one end of said hollow membrane fiber is connected the conduit means for receiving fluid from the micro-reservoir system and the other end is connected to a waste reservoir, said hollow membrane fiber having a porosity permitting passage therethrough of the target chemical from said test fluid while preventing passage therethrough of said sensing substance from said reagent fluid.

3. A sensing system according to Claim 1 wherein said reagent fluid includes a catalyst.

4. A sensing system according to Claim 3 wherein said catalyst is an enzyme which reacts with the target chemical to provide a heat that is proportional to the concentration of said target chemical.

5. A sensing system according to Claim 4 wherein said enzyme is selected from the group consisting of glucose oxidase, catalase, hexokinase, glucose dehydrogenase, cholesterol oxidase, lactase, urate oxidase, trypsin, apyrase, penicillinase, and mixtures thereof.

6. A sensing system according to Claim 1 wherein said micro-flow reservoir system includes a micro-flow reservoir comprising a fluid including a calibration compound.

7. A sensing system according to Claim 6 wherein said calibration compound is selected from the group consisting of hydrogen peroxide, catalase, glucose, target chemical, and mixtures thereof.

8. A sensing system according to Claim 2 wherein said hollow membrane fiber comprises a semipermeable dialysis membrane, and wherein the outer diameter of said hollow membrane fiber is in thermal communication with said sensing junctions.

9. A sensing system according to Claim 8 wherein said semipermeable dialysis membrane comprises a compound selected from the group consisting of acetate, polysulfone, polyacrylonitrile, cellulose, and mixtures thereof.

10. A sensing system according to Claim 1 wherein said thermopile comprises:

a thin film thermopile disposed upon a supporting substrate, said thin film thermopile including a plurality of pairs of thin film thermocouple junctions, each of said pairs of thermocouple junctions including a reference junction and a sensing junction electrically coupled in series connection with one another and spaced apart from one another, said reference junction and a said sensing junction within each pair of thin film thermocouple junctions creating a temperature-dependent voltage when said reference junction and said sensing junction are electrically coupled in series connection, said plurality of pairs of thermocouple junctions being electrically coupled in series connection with one another between first and second output terminals, said plurality of pairs of thermocouple also having a plurality of output terminals which are individually attached to a subset of reference junctions along the thermopile length in between the first and second terminals; and

wiring means coupled to each of said output terminals of said plurality of pairs of thin film thermocouple junctions for providing a voltage difference signal proportional to a difference in temperature measured proximate the sensing and reference junctions arising from reactions between the reagent fluid and target chemical.

11. A sensing according to Claim 1 wherein the at least one reservoir including reagent fluid comprises:

a containment enclosing a collapsible bag that is held at positive pressure, said collapsible bag housing the reagent fluid; and

a resistance tubing having an open end that is immersed in the reagent fluid to create sufficient fluidic resistance to control the flow rate of said reagent fluid through said open end of said resistance tubing.

12. A sensing arrangement according to Claim 1 wherein the sensor system comprises an optical cell.

13. A sensing arrangement according to Claim 12 wherein the reagent fluid comprises a reagent which exhibits a measurable change in optical properties upon contacting the target chemical.

14. A sensing arrangement according to Claim 13 wherein said optical property is at least one selected from the group consisting of absorbance, fluorescence, color change, and chemiluminescence.

15. A sensing arrangement according to Claim 13 wherein said sensing arrangement further comprising a gas permeable membrane fiber which is connected between said conduit and said sensor such that said gas permeable membrane fiber is in fluid contact with the test fluid.

16. A sensing arrangement according to Claim 13 further comprising a micro-flow reservoir comprising a sweep fluid,

a conduit connecting said reservoir comprising a sweep fluid to one end of a hollow membrane fiber which is in fluid contact with said test fluid wherein the other end is connected to the conduit comprising the reagent fluid down flow from said sensor.

17. A sensing arrangement according to Claim 16 wherein the sweep fluid is selected from the group consisting of water and saline.

18. A sensing arrangement according to Claim 16 wherein said reagent is mixed with said sweep fluid, thereby causing a measurable optical change.

19. A sensing arrangement according to Claim 18 wherein said optical property is at least one selected from the group consisting of absorbance, fluorescence, color change, and chemiluminescence.

ABSTRACT

The present invention provides an implantable sensing arrangement having long-term stability. The sensing arrangement utilizes microdialysis sampling techniques and includes a micro-flow reservoir having a reagent which reacts with a target chemical and a sensor connected to the micro-flow reservoir for detecting the reaction of the reagent and the target chemical. The sensor may include a thermopile or optical cell.

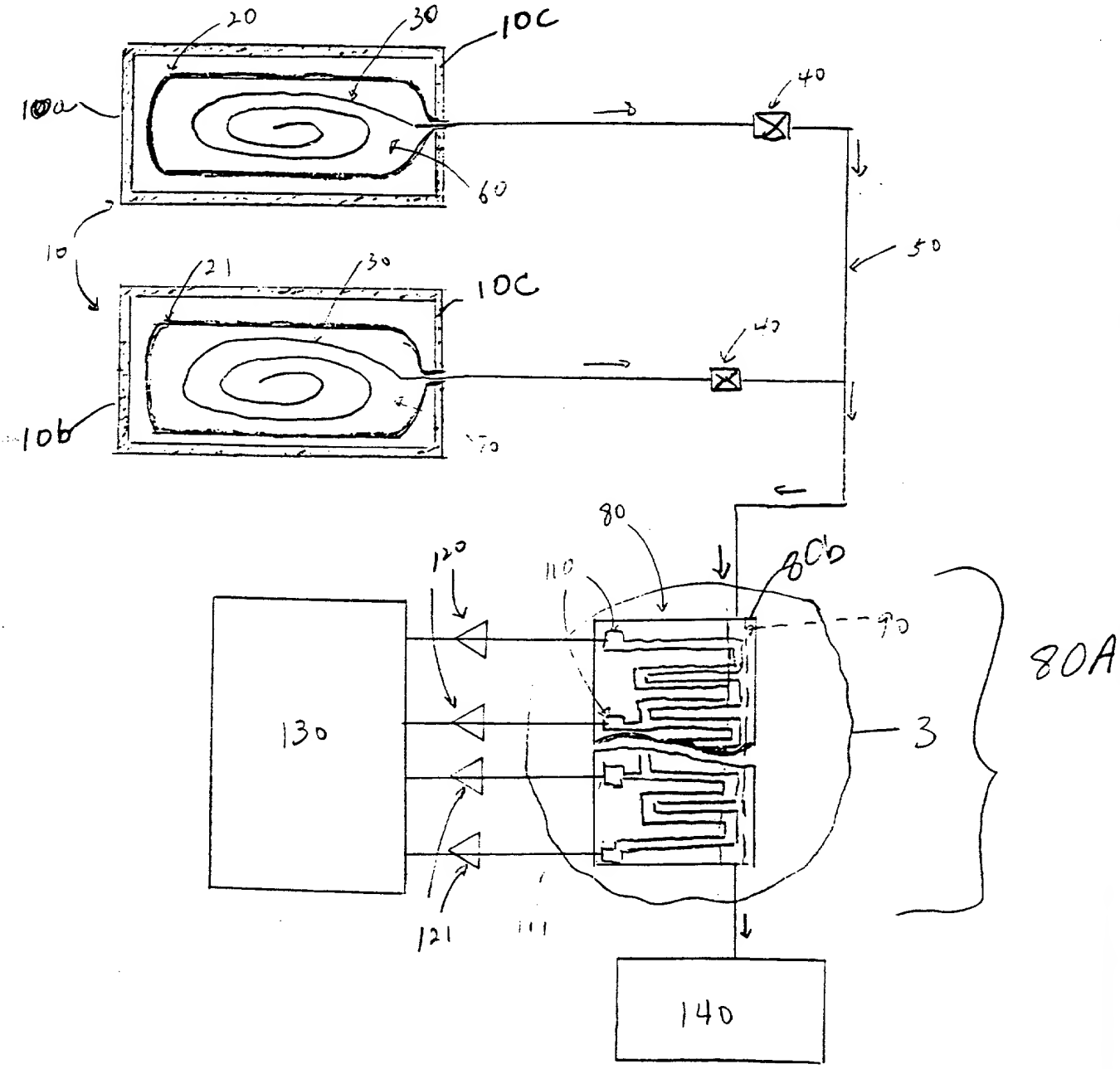


Fig. 1

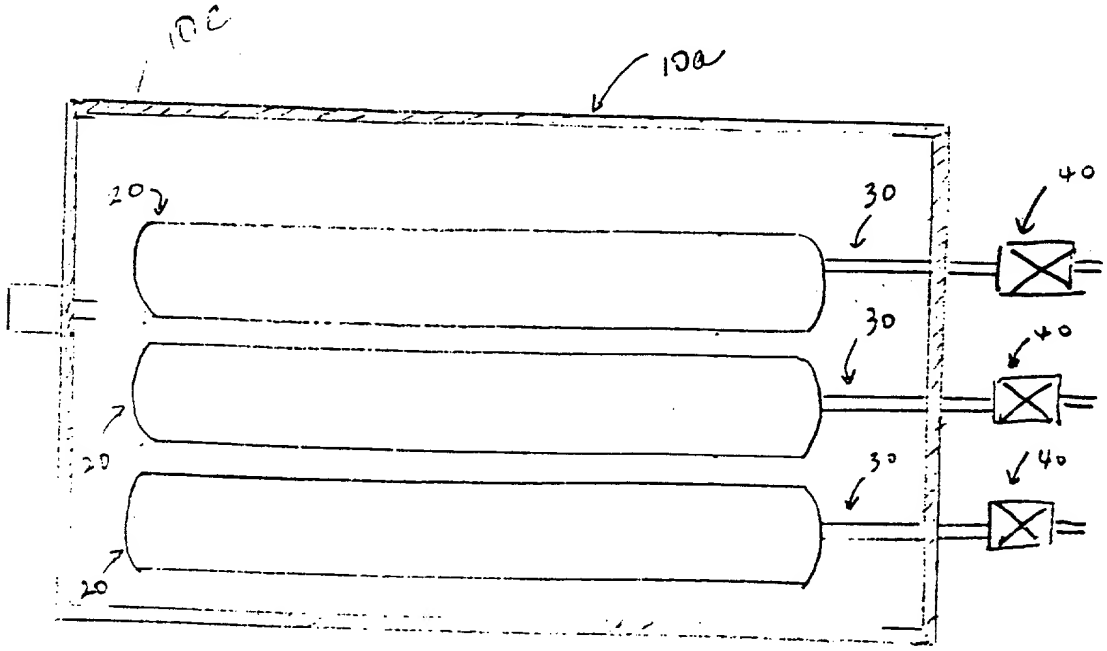


Fig. 2B

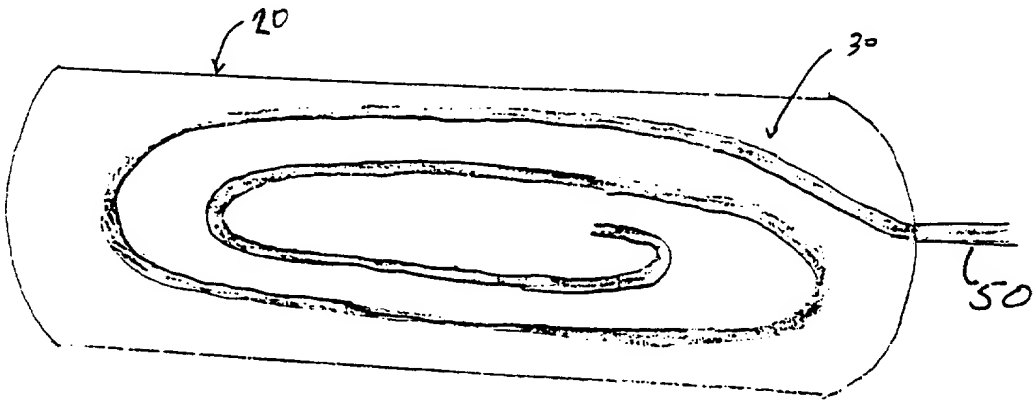


Fig. 2A

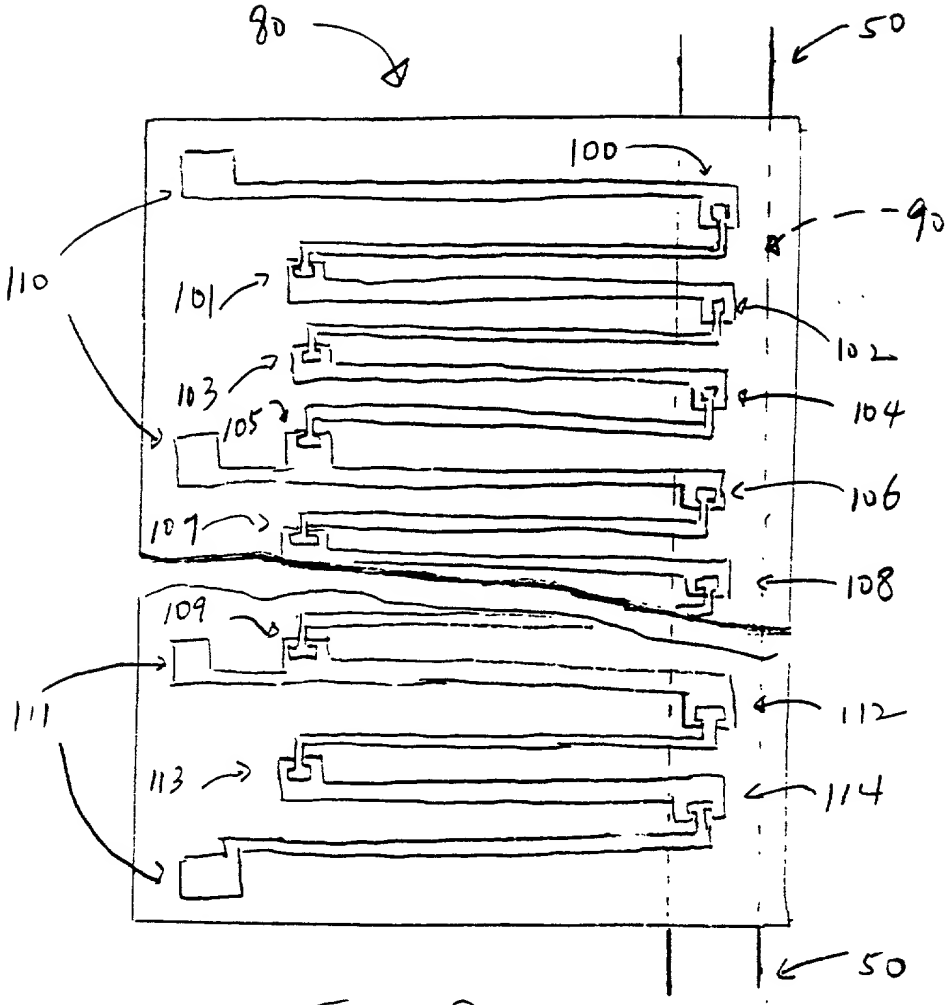


Fig. 3

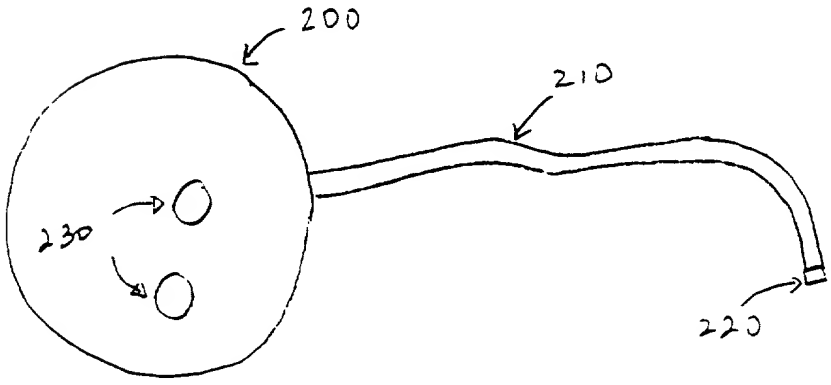


Fig 4

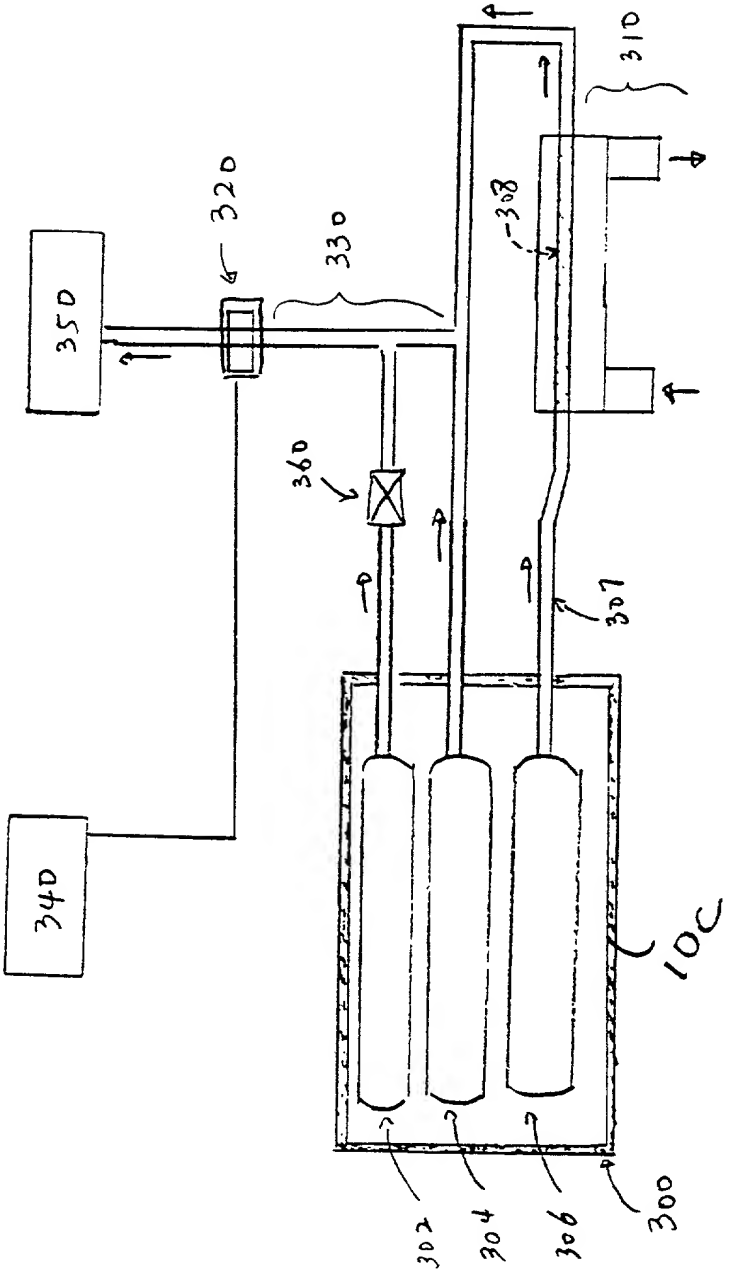


Fig 5

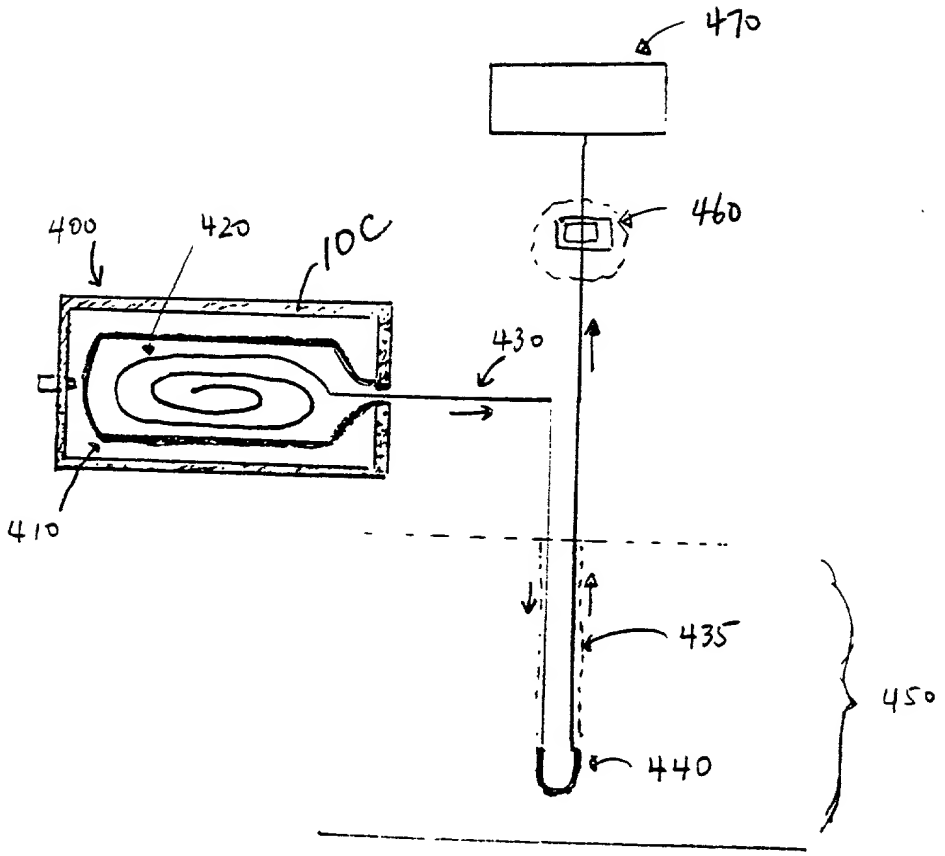


Fig. 6

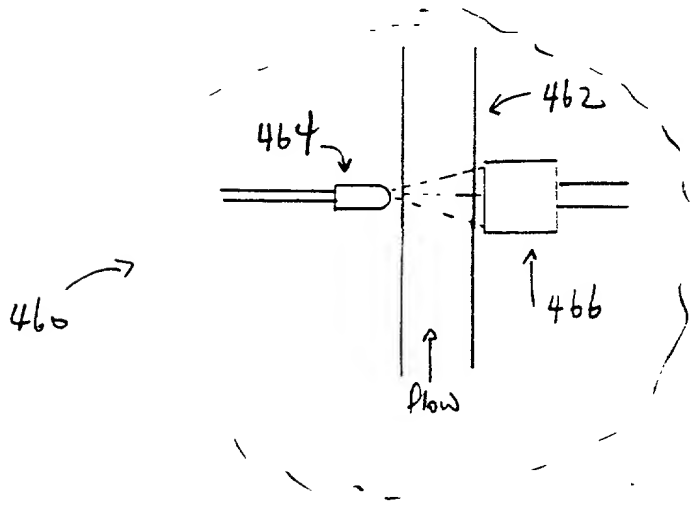


Fig 7.

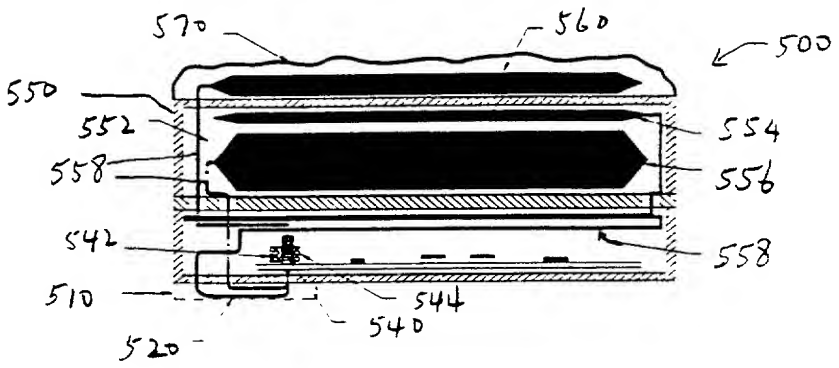


Fig. 8

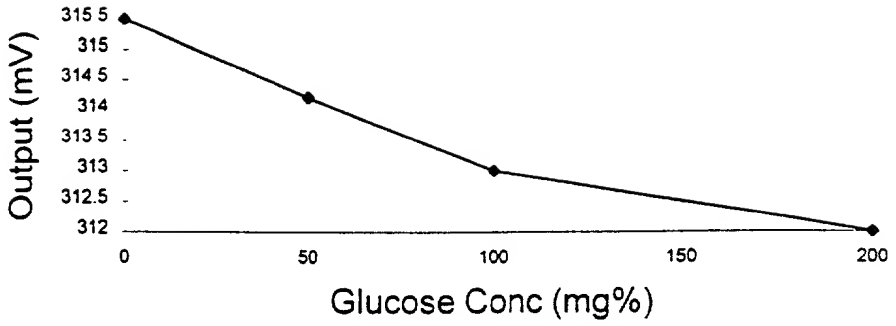


Fig 15

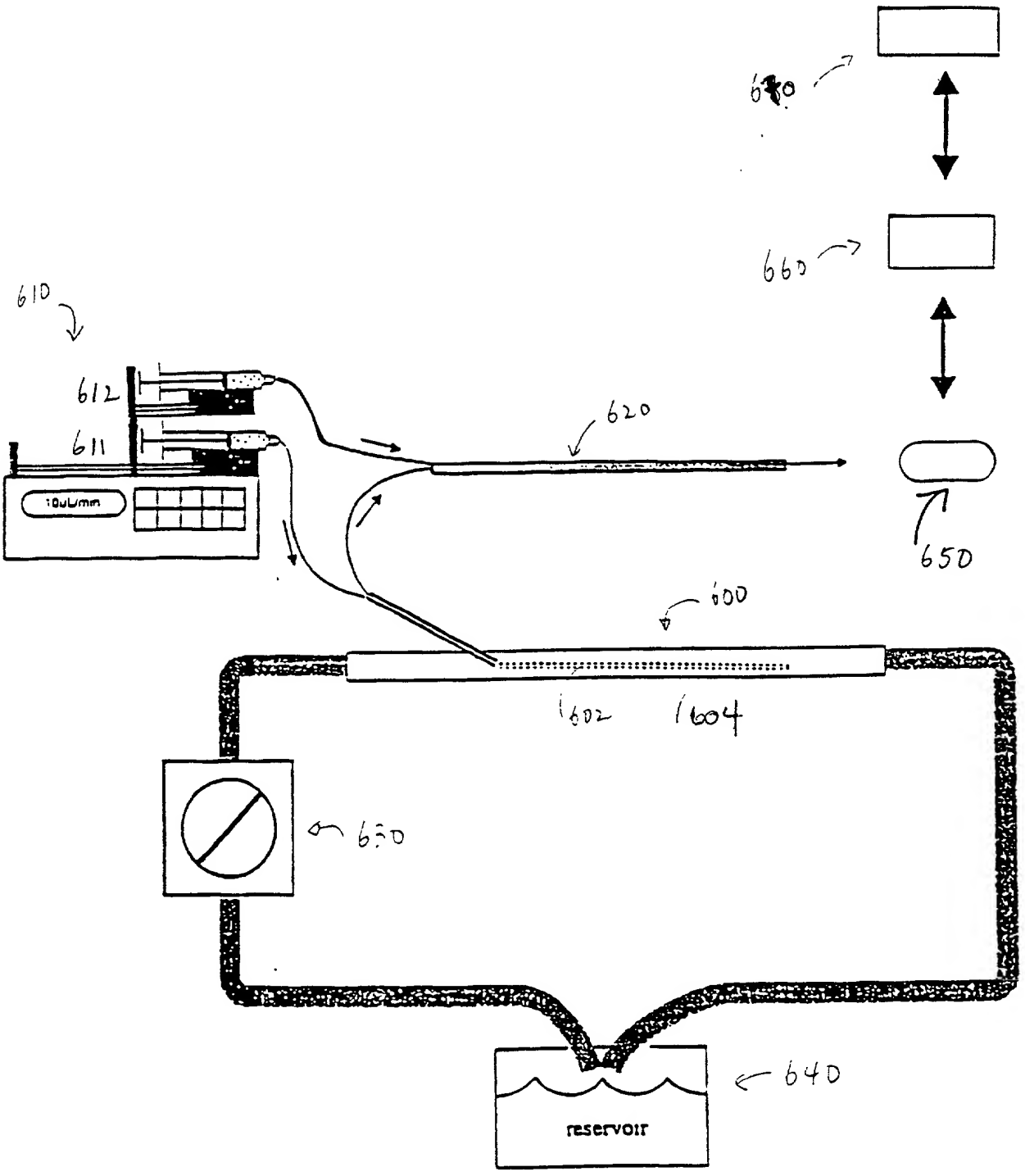


Fig. 9

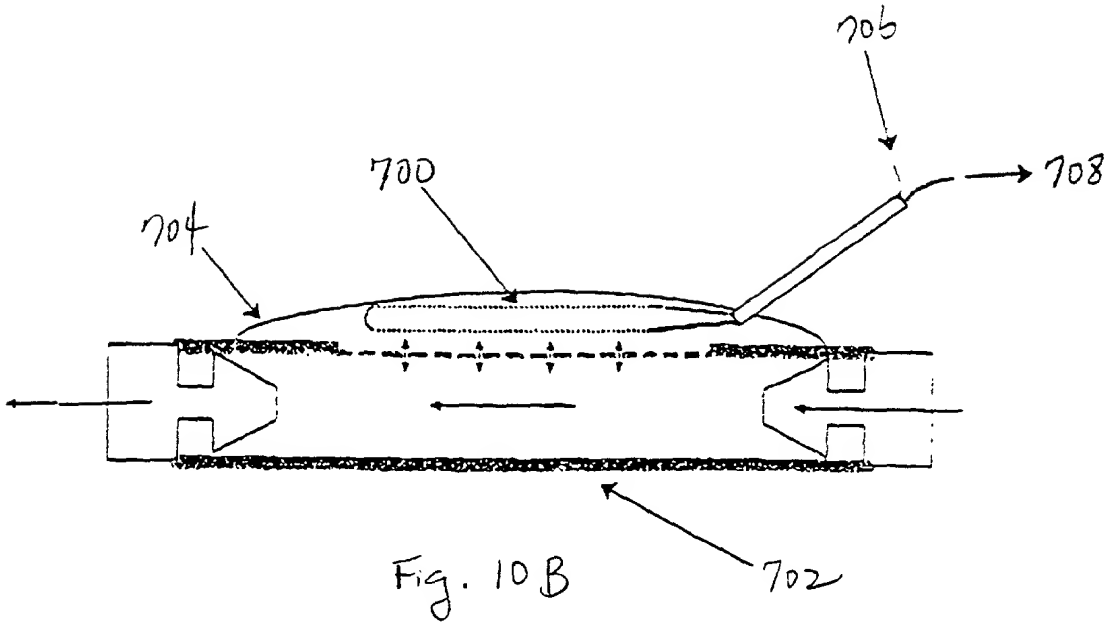
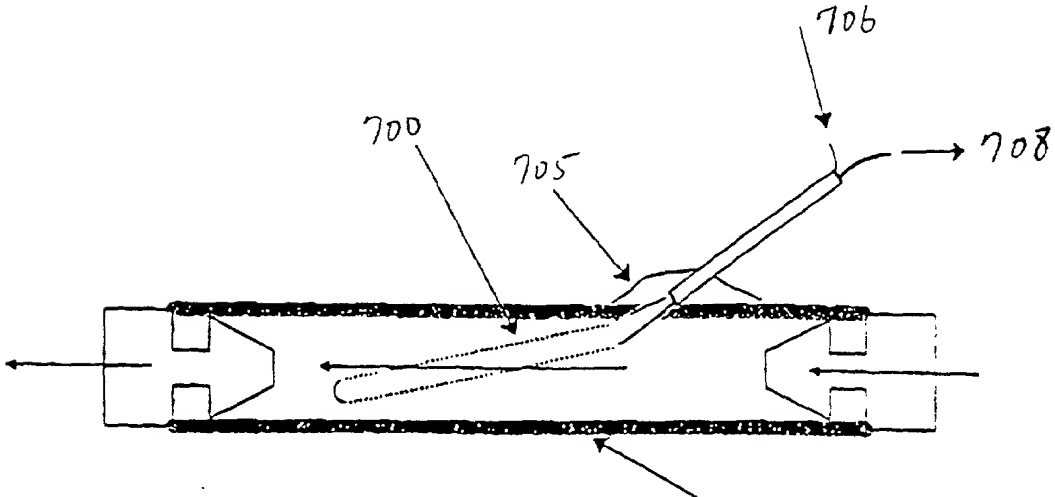


Fig. 10B



Fg 10A

Fig. 11

SC N 12

9/12

09/600947

Fig. 12

10/12

US/6009947

SCAN 12

Fig. 13

SCAN 12

11/12

09/600947

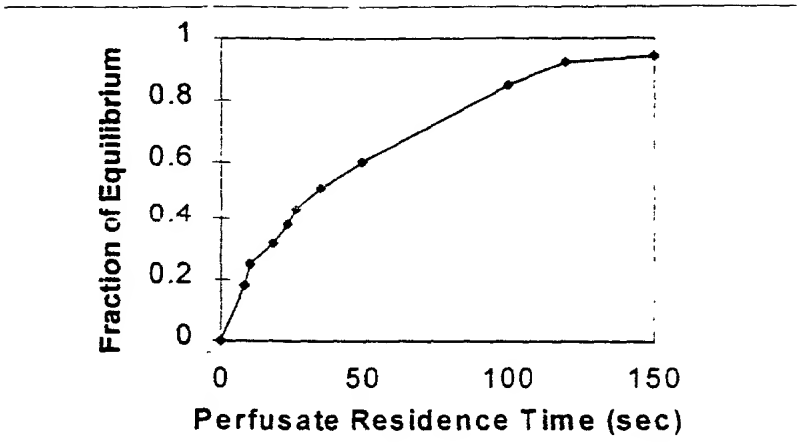


Fig 14

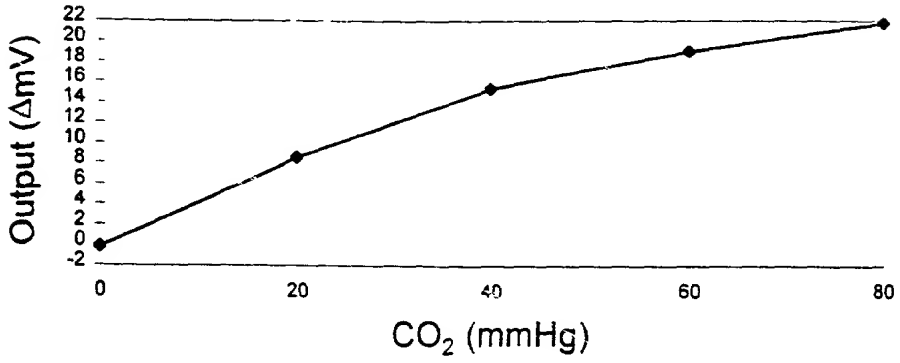


Fig 15

FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS`	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			

Check proper box(es) for any added page(s) forming a part of this declaration

[ ] Signature for ninth and subsequent joint inventors. Number of pages added _____.

[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added _____.

[ ] Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47. Number of pages added _____.

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHEMICAL SENSORS HAVING MICROFLOW SYSTEMS AND SENSING SYSTEMS THEREOF HAVING INCREASED STABILITY AND USEFUL LIFE"

This declaration is of the following type:

[] original
[] design
[X] national stage of PCT.
[] divisional
[] continuation
[] continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*

(a) [ ] is attached hereto.
(b) [ ] was filed on as Application Serial No. and was amended on *(if applicable)*.
(c) [X] was described and claimed in PCT International Application No. PCT/US99/02493 filed on February 4, 1999 and was amended on July 20, 1999.

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

[ ] In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

(d) [ ] no such applications have been filed.
(e) [ ] such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
COUNTRY APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
			[ ] YES NO [ ]
			[ ] YES NO [ ]
			[ ] YES NO [ ]
ALL FOREIGN APPLICATION[S], IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
			[ ] YES NO [ ]
			[ ] YES NO [ ]
			[ ] YES NO [ ]

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date
60/073,651	February 4, 1998
60/073,652	February 4, 1998
60/073,653	February 4, 1998

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836; Henry Tang, Reg. No. 29,705; Robert C. Scheinfeld, Reg. No. 31,300; John A. Fogarty, Jr., Reg. No. 22,348; Louis S. Sorell, Reg. No. 32,439; Rochelle K. Seide Reg. No. 32,300; Gary M. Butter, Reg. No. 33,841; Marta E. Delsignore, Reg. No. 32,689; and Lisa B. Kole, Reg. No. 35,225 of the firm of BAKER BOTTS L.L.P., with offices at 30 Rockefeller Plaza, New York, New York 10112, as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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